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Implantation: molecular basis of embryo-uterine dialogue

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ABSTRACT Implantation is a complex developmental process that involves an intimate "cross-talk" between the embryo and uterus. Synchronized development of the embryo to the blastocyst stage and differentiation of the uterus to the receptive state are essential to this process. Successful execution of the events of implantation involves participation of steroid hormones, locally derived growth factors, cytokines, transcription factors and lipid mediators. Using gene-targeted mice and a delayed implantation model, our laboratory has been exploring potential interactions among steroid hormones, growth factors, cytokines and prostaglandins in this process. This review article highlights some of our recent observations on the roles of estrogen, catecholestrogen, the EGF family of growth factors, leukemia inhibitory factor and cyclooxygenase-2 derived prostaglandins and their interactions in embryo-uterine "cross-talk" during implantation.

KEY WORDS: *Embryo, implantation, growth factors, cytokines, prostaglandins.*

Procreation, both sexual and asexual, is a fundamental and evolving process to sustain life in this universe. Thus, understanding this process has been the inspiration of investigators for centuries. Sexual procreation in higher eukaryotes, especially in mammals, is inferior to asexual procreation in prokaryotes and in some lower eukaryotes with respect to shear number of progeny produced. Mammalian reproduction on the other hand, is more complex and highly regulated for the propagation of superior offspring to carry on the task of procreation. In sexual reproduction, the fate of the union of a sperm with an egg marks the beginning of embryonic development or death. A successful union leads to fertilization and subsequent development, while failure to achieve such a union leads to the demise of the gametes. In most eutherian mammals, the one-cell fertilized egg, here termed the embryo, undergoes mitotic cell division ultimately forming a blastocyst with two distinct cell populations, the inner cell mass (ICM) and a layer of trophectoderm cells surrounding the ICM (Gardner and Papaioannou, 1975). The ICM forms the embryo proper and trophectoderm cells first make the physical and physiological contact with the maternal uterine luminal epithelium to initiate the process of implantation, leading to the establishment of pregnancy (reviewed in Dey, 1996). A substantial embryo loss due to preimplantation death is common to many mammals, and it is considered a selection process that leads to the survival of superior embryos for implantation. Also, the failure of the events during and immediately following implantation often adds to the poor pregnancy rate in eutherian mammals (reviewed in Cross *et al.*, 1994). Therefore, understanding and unraveling the secrets of preimplantation embryo development and implantation in the maternal uterus has been a challenge to the investigators in the field aiming to alleviate the problems of infertility, ensuring the birth of quality offspring, or developing novel contraception to restrict world population.

The current state of the knowledge of preimplantation and implantation physiology is the result of accumulation of scientific observations gathered by many pioneers through the years. One of the pioneers in this field is Ann McLaren whose seminal observations have formed the basis of further investigation in the field. This review article on implantation is dedicated to Ann McLaren to show our tribute for her contribution in the field of preimplantation and implantation biology.

Embryonic development to the blastocyst stage and uterine differentiation to the receptive environment are essential to the establishment of the embryo-uterine "cross-talk" that leads to the initiation and progression of successful implantation. A complex series of interactive molecular events is associated with this process. However, the precise sequence and details of these molecular events have not yet been defined. Since the process of implantation is complex and varies across species, a unified theme has not yet been formulated, resulting in an accumulation of literature often with contradictory interpretations. Thus, it is an arduous task to write an overview on the molecular basis of embryo-uterine interactions during implantation that could be

Abbreviations used in this paper: COX, cyclooxygenase-2; LIF, leukemia inhibitory factor.

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relevant to mammals in general. This review focuses on the molecular basis of implantation based on the information generated with mice primarily from our laboratory. However, a few of the distinct differences with other species are highlighted.

Preimplantation embryo development

Preimplantation embryo development to the blastocyst stage, which requires activation of the embryonic genome, is essential to the process of implantation. This maternal-zygotic transition occurs at the 2-cell stage in mice and other rodents, between the 8- and 16-cell stage in cows and sheep, and between 4- and 8-cell stage in humans (reviewed in Artley *et al.,* 1992). Once the embryonic genome is activated, the embryo grows rapidly to form a blastocyst. At this stage, embryos mature and escape from their zona pellucida to achieve implantation competency. The fully expanded blastocyst is composed of three cell types: (i) the outer polarized epithelial trophectoderm, (ii) the primitive endoderm, and (iii) the pluripotent ICM. The ICM provides the future cell lineages for the embryo proper (Hogan *et al.,* 1994a; McLaren, 1982a). However, no ICM is identifiable in marsupial blastocysts; they appear as a hollow ball of cells with similar morphological characteristics. It is also not known which cells are programmed to form the embryo proper (reviewed in McLaren, 1982a).

In mice and rats, normal embryonic development to the blastocyst stage within the reproductive tract requires the presence of ovarian estrogen and progesterone (McLaren, 1971). There is a decrease in the number of embryos and number of cells per embryo in the absence of these hormones (Bowman and McLaren, 1970); treatment with estrogen and progesterone reverses these defects (Roblero and Garavagno, 1979). Since there is no evidence that estrogen and/or progesterone act directly on the preimplantation embryo, embryonic development is considered to depend on growth-promoting factors originating from the reproductive tract under the influence of these hormones. However, normal development in defined media in cultures suggests that preimplantation embryos themselves produce the growth-promoting factors (reviewed in Paria and Dey, 1990). This is consistent with the expression of several growth factors, cytokines and their receptors in the embryo and uterus, as well as beneficial effects of these factors on embryonic development and functions (reviewed in Carson *et al.,* 2000; Paria and Dey, 1990; Paria *et al.,* 2000; Stewart and Cullinan, 1997).

Uterine preparation for blastocyst implantation

The molecular mechanism(s) that renders the uterus receptive to blastocyst implantation is not fully understood. The “window” of uterine receptivity is defined as the limited time when the uterine environment is conducive to blastocyst implantation. The synchronized development of the embryo to the blastocyst stage and the differentiation of the uterus to the receptive state are critical to this process (Dey, 1996; Paria *et al.,* 1993; Psychoyos, 1973). Although various aspects of molecules associated with this process are extensively being explored, a comprehensive understanding of the subject is still very limited.

Uterine differentiation to support embryo development and implantation is coordinated by progesterone and estrogen in a spatiotemporal manner (Huet-Hudson *et al.,* 1989). In the rodent, the first conspicuous sign that the implantation process has been initiated is an increased uterine vascular permeability at the sites of blastocyst apposition (Psychoyos, 1973). This is demarcated by discrete blue bands along the uterus after an intravenous injection of a blue dye solution (Paria *et al.,* 1993; Psychoyos, 1973). This heightened, localized vascular permeability coincides with the initial attachment reaction between the luminal epithelium and the blastocyst (Enders and Schlafke, 1967) and is considered one of the earliest prerequisites for implantation (Psychoyos, 1973). In the mouse, these events occur in the evening (2200-2300 hour) of day 4 (day 1=vaginal plug) of pregnancy (Das *et al.,* 1994). The attachment reaction is followed by localized decidualization of the stroma and apoptosis of the luminal epithelium at the sites of implantation (Parr *et al.,* 1987), facilitating invasion of the trophoblast cells through the underlying basement membrane (Schlafke and Enders, 1975). The blastocyst attachment with the uterine luminal epithelium and its subsequent apoptosis is illustrated in Fig. 1. These processes are accompanied by remodeling of the extracellular matrix and angiogenesis in the stromal bed (reviewed in Carson *et al.,* 2000; Dey, 1996). The molecular mechanisms by which increased localized vascular permeability and angiogenesis occur at the implantation site are still poorly understood.

The requirement of ovarian estrogen is species-specific. While estrogen is essential for preparation of the progesterone-primed uterus for implantation in mice and rats, progesterone alone is capable of initiating implantation in species, such as the hamster, guinea pig, pig and rabbit (Deanesly 1960; Kwun and Emmens, 1974; Orsini and Meyer, 1962; Perry *et al.,* 1973). Although progesterone is an absolute requirement for implantation, it is not yet clear whether estrogen is a requirement for implantation in primates (de-Ziegler *et al.,* 1998; Meyer *et al.,* 1969; Ravindranath and Moudgal, 1987; Yoshinaga, 1994). Most of our understanding about the requirement of ovarian steroid hormones for the preparation of the uterus for implantation is based on studies in mice and rats. In the adult mouse, estrogen stimulates proliferation of uterine epithelial cells, and progesterone stimulates stromal cell proliferation. These two steroids together further potentiate stromal cell proliferation (Huet-Hudson *et al.,* 1989). Similar hormonal stimulation of cell-specific proliferation occurs in the preimplantation uterus. Preovulatory ovarian estrogen secretion induces proliferation of the luminal and glandular epithelial cells during the first two days of pregnancy. On the third day, progesterone from newly formed corpora lutea initiates stromal cell proliferation which is further potentiated by preimplantation estrogen secretion on day 4 of pregnancy (the day of implantation) (Huet-Hudson *et al.,* 1989). Uterine sensitivity to implantation in response to ovarian steroids is programmed into three phases: prereceptive, receptive, and nonreceptive. The prereceptive uterus on day 3 of pregnancy becomes receptive on day 4 under the influence of rising progesterone and preimplantation ovarian estrogen secretion (Dey, 1996; Paria *et al.,* 1993). Blastocysts implant only in the receptive uterus, which spontaneously progresses to the nonreceptive phase. The preimplantation estrogen secretion is absolutely necessary for the establishment of uterine receptivity in the rat and mouse (McCormack and Greenwald, 1974; Paria *et al.,* 1993; Psychoyos, 1973). Although the hormonal requirement for uterine preparation and implantation is different in various species, the molecular basis of these differences is not yet known and needs to be addressed. However, the preimplantation embryo as a source of estrogen

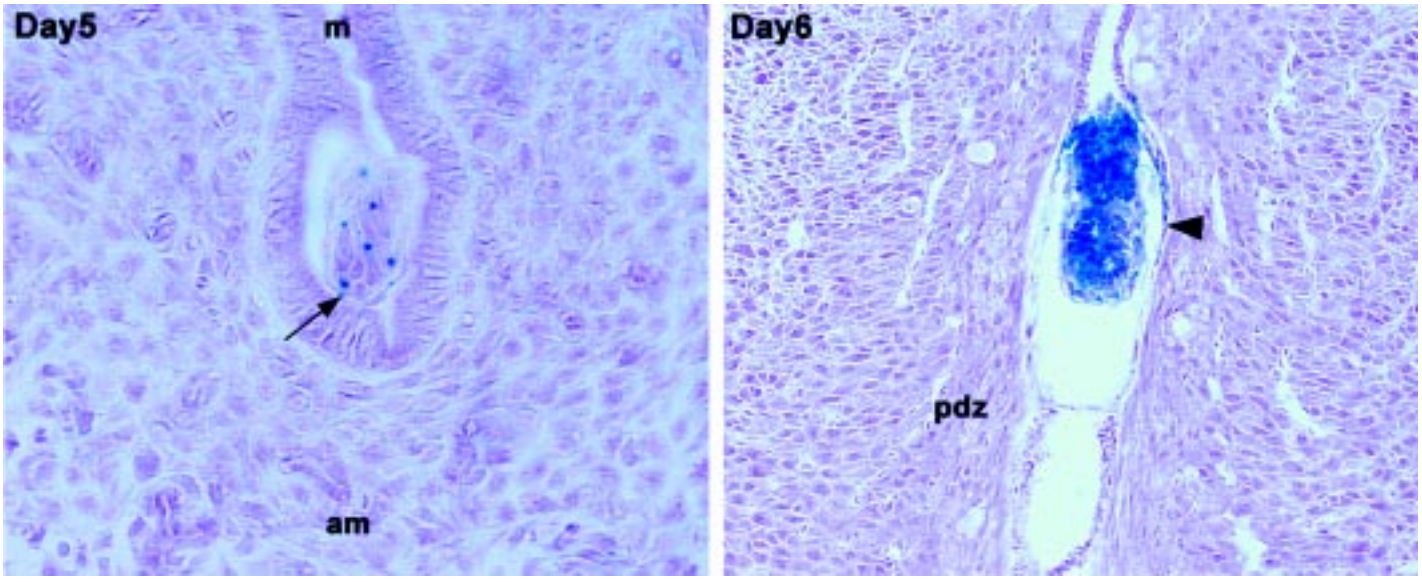

Fig. 1. Photomicrographs of cross sections of implantation sites on days 5 and 6 of pregnancy in the mouse. *Arrow and arrowhead indicate antimesometrial attachment of the blastocyst trophectoderm to the luminal epithelium on day 5 and the loss of uterine epithelium surrounding the implanting blastocyst on day 6, respectively. Day 4 blastocysts from ROSA 26 donors were transferred into the uteri of day 4 pseudopregnant CD-1 recipients and implantation sites were monitored by the blue dye method (Psychoyos, 1973). Implantation sites were fixed, sectioned and processed for LacZ staining (Hogan et al., 1994b). ROSA 26 mice ubiquitiously express the β-galactosidase transgene (Friedrich and Soriano, 1991). Note nuclear LacZ staining in embryonic cells. am, antimesometrial pole; m, mesometrial pole; pdz, primary decidual zone.*

influencing implantation in the species not requiring ovarian estrogen can not be ruled out. Indeed, rabbit and pig blastocysts have an estrogen-synthesizing capacity, which is likely to participate in implantation (Hoversland *et al.,* 1982; Perry *et al.,* 1973). On the other hand, preimplantation mouse embryos do not possess the machinery for producing estrogen (Stromstedt *et al.,* 1996). Furthermore, normal implantation of mouse or human blastocysts carrying a nonfunctional aromatase gene suggests that embryonic estrogen is not required for implantation in these species (Fisher *et al.,* 1998; Morishima *et al.,* 1995). It is still unclear whether embryonic estrogen is required for the initiation of implantation in hamsters and primates. Further investigation is warranted to understand the molecular basis of these differential roles of estrogen and progesterone in the preparation of the receptive uterus and implantation among various species. In this respect, the delayed implantation model in certain species provides an opportunity to dissect out the distinctive and overlapping functions of estrogen and progesterone in the uterus as it relates to implantation.

Delayed implantation

As discussed above, in the mouse preimplantation ovarian estrogen secretion on the morning of day 4 of pregnancy is essential for the attachment reaction that occurs in the evening of this day. However, the implantation process is deferred if ovaries are removed on day 4 of pregnancy before the secretion of preimplantation ovarian estrogen. This condition, known as delayed implantation, can be maintained for several days by daily progesterone treatment (Psychoyos, 1973; Yoshinaga and Adams, 1966). Under this condition, the uterus remains in a neutral state (analogous to the prereceptive phase) and blastocysts undergo dormancy. Delayed implantation can be terminated by an injection of estrogen to initiate implantation with blastocyst activation (McLaren, 1971; Paria *et al.,* 1993; Psychoyos, 1973). Delayed implantation can also be induced by other means including hypophysectomy and interference with hypothalamic functions (Bindon, 1969). Lataste in 1891 first described the natural suckling-induced delayed implantation (facultative) in mice and rats that had been mated during the post-partum estrus. This type of naturally-occuring delayed implantation varies between strains of mice and rats and even between individuals within a particular strain (Gidley-Baird, 1981). However, dormant blastocysts become activated and undergo implantation upon termination of the suckling stimulus by removal of pups. In many wild animals including mustelids and mursupials, delayed implantation is obligatory and seasonal (reviewed in McLaren, 1982b). Interestingly, delayed implantation does not occur in hamsters, guinea pigs, rabbits, and pigs. However, it is not known whether this condition ever occurs in primates. Although estrogen is essential to terminate delayed implantation in the progesterone-primed mouse or rat uterus, the mechanism by which estrogen directs this function is not clearly understood.

McLaren's group using labeled substrates, first showed that dormant mouse blastocysts are metabolically less active during delayed implantation than they are during normal implantation or during reactivation after estrogen treatment (Menke and McLaren, 1970). This was later confirmed by other investigators (Weitlauf, 1974). This observation by McLaren's laboratory created a tremendous interest in the field and led to the proposal that the lower metabolic rate of dormant blastocysts is the result of the presence of an inhibitor in the delayed uterus (Weitlauf, 1974; 1978). On the other hand, Surani (1975) suggested that the disappearance of a stimulatory factor from the uterus during the delay is the cause for blastocyst dormancy. Although dormant blastocysts gain metabolic competence *in vitro* (McLaren, 1973) suggesting the pres-

ence of an inhibitor, the subject still remains unresolved. We raised the question of whether metabolic competency is similar to implantation competency of the blastocyst. Our work, as described below, suggests that the blastocyst's state of activity is also an important factor in the initiation of implantation in the receptive uterus.

Blastocyst state of activity and uterine receptivity determine the window of implantation

The uterine receptivity for blastocyst implantation lasts only for a limited period, followed by spontaneous progression to the non-receptive phase when the uterine milieu becomes hostile to blastocyst survival. In rats and mice, uterine receptivity lasts for about 24-36 h (Paria *et al.,* 1993; Psychoyos, 1973), while this window appears to last longer in primates (reviewed in Yoshinaga, 1994). In the past, it was considered that the uterine receptivity was the primary determinant for successful implantation regardless of the blastocyst's state of activity. However, our recent work, using blastocyst transfer experiments in delayed implanting mice, have demonstrated that the blastocyst's state of activity is also an important factor in defining the "window" of implantation in the receptive uterus (Paria *et al.,* 1993). For example, dormant blastocysts transferred into uteri of progesterone-treated delayed recipients implanted only if they were transferred within one hour of an estrogen injection to the recipients. In contrast, day 4 normal or *in utero* estrogen-activated blastocysts successfully implanted even when transferred at 16 h after estrogen treatment to similar recipients. In contrast, the transfer of dormant blastocysts or *in vitro* cultured "metabolically-competent" dormant blastocysts failed to implant if transferred beyond one hour of the estrogen treatment of the progesterone-primed recipients. These results suggested a clear difference between *in vitro* activated blastocysts and those activated by estrogen *in utero* and that implantation occurs when the window of uterine receptivity coincides with the blastocyts's state of activity. Similar transfer experiments also indicated that the progesterone-treated uterus in the presence of estrogen rapidly, but transiently, generates a factor(s) that activates the dormant blastocyst for implantation. Moreover, our results suggested that estrogen-induced uterine receptivity and blastocyst activation are two separate events, although it was not known whether these events were executed by the same or different factors (Paria *et al.,* 1993).

Coordinated effects of estrogen and catecholestrogen on two targets direct implantation

As stated above, estrogen is essential for differentiation of the uterus to the receptive state and activation of the blastocyst for implantation in a progesterone-primed mouse uterus. However, it was difficult to delineate the dual role of estrogen on these two targets. Our work has suggests that estrogen action in implantation involves catecholestrogens which are active metabolites of primary estrogens (Paria *et al.,* 1998). Catecholestrogens, like primary estrogens, can function via classical nuclear estrogen receptors and/or membrane receptors, and are formed in various tissues including the uterus and embryo (reviwed in Paria *et al.,* 1998). Furthermore, catecholestrogens, but not estrogen, can stimulate prostaglandin (PG) synthesis in blastocysts and endometrial cells *in vitro*. Because PGs are important for embryo development and implantation, we suspected that a catecholestrogen produced from the primary estrogen in the progesterone-primed uterus initiates blastocyst activation in a paracrine manner (reviewed in Paria *et al.,* 1998). Indeed, we have recently demonstrated that while estradiol-17β (E_2, a primary estrogen) fails to activate dormant blastocysts *in vitro*, 4-hydroxy-estradiol-17β (4-OH-E_2, a catecholestrogen) is effective in this response. Furthermore, an antiestrogen fails to block 4-OH-E_2 mediated activation of dormant blastocysts *in vitro*, suggesting a unique role of this estrogen in influencing blastocyst functions. To examine the site-specific actions of primary estrogen and catecholestrogen in uterine preparation and blastocyst activation for implantation, respectively, 2-fluoroestradiol-17β (2-Fl-E_2) was used. Although 2-

TABLE 1

GENES INVOLVED IN PREIMPLANTATION AND IMPLANTATION BIOLOGY: GENE-TARGETED MOUSE MODELS AND THEIR REPRODUCTIVE PHENOTYPES

Gene	Function	Reproductive Phenotypes	References
E-cadherin	Junctional protein	Failure of blastocyst formation	Larue *et al.,* 1994
FGF-4	Growth factor	Defective proliferation of inner cell mass	Feldman *et al.,* 1995
erbB1	Receptor for EGF-like growth factors	Postimplantation failure depending on the genetic background	Threadgill *et al.,* 1995; Miettinen *et al.,* 1995; Sibilia and Wagner, 1995
α-catenin	Adhesion molecule	Disruption of blastocyst trophectoderm	Torres *et al.,* 1997
IGF-I	Growth factor	Defective ovarian and uterine functions	Baker *et al.,* 1996
Wnt7a	Cell signaling	Female sterility	Parr and McMahon, 1995
COX-2	Prostaglandin synthesis	Ovulation, fertilization and implantation defects	Lim *et al.,* 1997
ERα	Nuclear estrogen receptor	Female sterility. Various aspects of reproductive failures	Lubahn *et al.,* 1993
PR	Nuclear progesterone receptor	Female sterility. Various aspects of reproductive failures	Lydon *et al.,* 1995
LIF	Cytokine	Implantation and decidualization failures	Stewart *et al.,* 1992
IL-11Rα	IL-11 receptor	Defective decidualization	Robb *et al.,* 1998
Hmx3	Homeobox transcription factor	Defective implantation	Wang *et al.,* 1998
Hoxa-10	Homeobox transcription factor	Implantation and decidualization defects	Benson *et al.,* 1996; Lim *et al.,* 1999b
Hoxa-11	Homeobox transcription factor	Implantation and decidualization defects	Hsieh-Li *et al.,* 1995; Gendron *et al.,* 1997
$cPLA_2$	Arachidonic acid release	Small litter size. Cause unknown	Uozumi *et al.,* 1997; Bonventre *et al.,* 1997
CSF-1	cytokine	Reduced female fertility	Pollard *et al.,* 1991

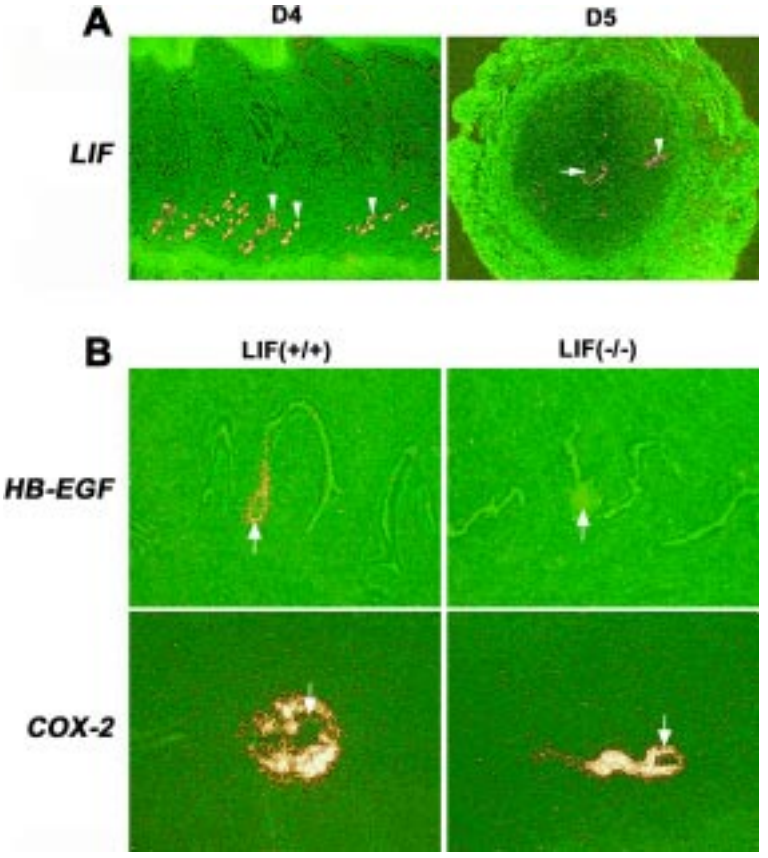

Fig. 2. *In situ* hybridization of *LIF, HB-EGF* and *COX-2* mRNAs in the mouse uterus on days 4 and 5 of pregnancy. (A) *uterine LIF mRNA localization in wild-type mice at 09.00 h on days 4 and 5. Note the expression of LIF mRNA in the glandular epithelium on day 4 and in stromal cells surrounding the implanting blastocyst on day 5.* **(B)** *Photomicrographs of longitudinal sections of LIF(+/+) and LIF(-/-) mouse uteri at 18.00 h on day 4 and 09.00 h on day 5 of pregnancy showing HB-EGF and COX-2 expression, respectively. Note HB-EGF mRNA expression in the luminal epithelial cells surrounding the blastocyst at 18.00 h on day 4 in LIF(+/+) mice; similar expression is absent in LIF(-/-) mice. COX-2 mRNA, which is normally expressed in both the luminal epithelial and stromal cells surrounding an implanting blastocyst at 09.00 h on day 5 in LIF(+/+) mice, is aberrantly expressed in LIF(-/-) mice; the expression is only present in the luminal epithelium. Arrows and arrowheads indicate blastocysts and uterine glands, respectively. (Reprinted with permission from Ref. Song et al., 2000).*

Fl-E_2 is a poor substrate for and a potent inhibitor of estrogen 2/4-hydroxylase that converts primary estrogens to catecholestrogens, it is equipotent to E_2 with respect to cell-specific uterine growth and gene expression (reviewed in Paria *et al.,* 1998). However, 2-Fl-E_2 even at a dose of 75 ng/mouse failed to induce implantation in progesterone-primed delayed implanting mice, whereas E_2 at this dose or even at 10 ng/mouse induced implantation in similarly treated delayed implanting mice. These results suggested that 2-Fl-E_2 prepared the uterus but failed to activate the dormant blastocyst, possibly because of its failure to form catecholestrogens. This is consistent with the finding that day 4 normal blastocysts, or dormant blastocysts cultured in the presence of 4-OH-E_2 did implant when they were transferred into uteri of progesterone-primed delayed recipients receiving an injection of 2-Fl-E_2. However, dormant blastocysts cultured in the presence of E_2 failed to implant in similarly treated mice. These results suggest that dormant blastocysts become implantation competent when they are cultured with 4-OH-E_2, but not with E_2. Collectively, our results demonstrate that primary estrogen participates in the preparation of the progesterone-primed uterus to the receptive state in an endocrine manner, whereas its metabolite catecholestrogen produced in the uterus mediates blastocyst activation for implantation in a paracrine manner, suggesting target-specific effects of estrogens during implantation. However, the molecular mechanisms by which these estrogens mediate their effects are not clearly understood. There is increasing evidence that the steroid hormones interact with the local factors at the targets to initiate the process of embryo-uterine interactions during implantation.

Potential molecular signaling during implantation

Recent progress in genetics and molecular biology has remarkably increased our knowledge regarding the roles of locally derived growth factors, cytokines, homeotic gene products and lipid mediators during implantation and decidualization. These factors are produced by the uterus or embryo independently or cooperatively under the influence of steroid hormones. Due to the space limitation, this review will discuss only a few of these local factors that are critical to the processes of implantation and decidualization. However, roles of various other genes in preimplantation and implantation phenomena as evident from gene targeting experiments are presented in Table 1.

The EGF family of growth factors

Among various growth factors, the EGF family of growth factors is considered to be involved in implantation. This family includes EGF itself, TGF-α, HB-EGF, amphiregulin, betacellulin, epiregulin and neuregulins (Das *et al.,* 1997; Paria *et al.,* 2000). Although TGF-α and amphiregulin, but not EGF, are expressed in the mouse uterus during the periimplantation period (Das *et al.,* 1997; Paria *et al.,* 2000), they do not appear to be essential for implantation since mice deficient of these genes are fertile (Luetteka *et al.,* 1993;1999). However, it is possible that the other members of the EGF family compensate for the loss of these members. Both *epiregulin* and *betacellulin* are also expressed in the luminal epithelium and stroma at the sites of blastocyst apposition during implantation (Das *et al.,* 1997). However, their roles in implantation are not yet clearly understood.

HB-EGF appears to have an expression pattern highly relevant to the implantation process. In the mouse, it is induced exclusively in the luminal epithelium at the site of blastocyst apposition 6-7 hours before the initial attachment reaction (Das *et al.,* 1994; Paria *et al.,* 2000). Since *HB-EGF* is not expressed in the uterus during the delayed implantation, it is considered that a signal is transmitted from the active blastocyst to the luminal epithelial cells for the expression of *HB-EGF* at the site of subsequent implantation. *In vitro* experiments have shown that soluble HB-EGF stimulates proliferation, zona-hatching, trophoblast outgrowth and ErbB1 phosphorylation of murine blastocysts (Das *et al.,* 1994). Furthermore, cells expressing the transmembrane form of HB-EGF ad-

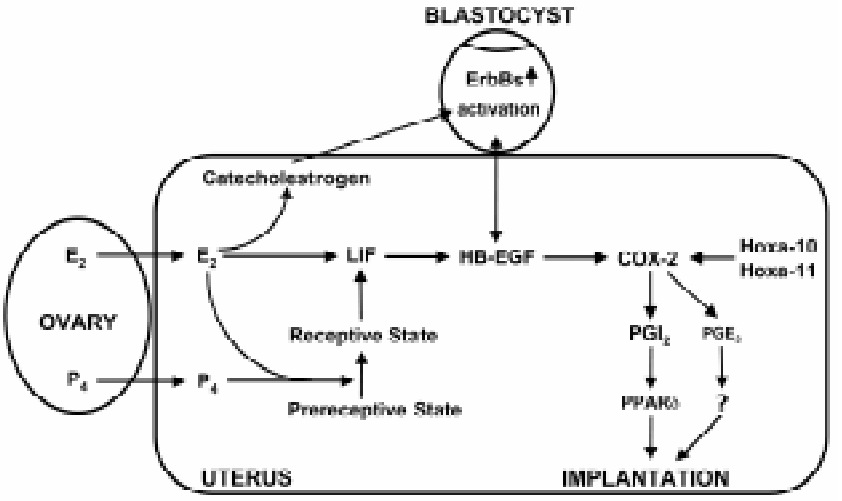

Fig. 3. Representation of a potential molecular cascade of events in the process of implantation in the mouse.

here to active mouse blastocysts expressing ErbB1 or ErbB4 (Rabb *et al.,* 1996; Paria *et al.,* 1999). There is also *in vitro* evidence that HB-EGF conjugated to *pseudomonas* exotoxins targets blastocysts by binding to erbB4 and HSPG (Paria *et al.,* 1999). However, these interactions of HB-EGF are absent in dormant blastocyst which do not express *erbB1*, *erbB4* or HSPG. HB-EGF also appears to be involved in human implantation, since its expression is maximal during the late secretary phase (cycle days 20-24) when the endometrium becomes receptive for implantation (Leach *et al.,* 1999; Yoo *et al.,* 1997). HB-EGF has been shown to be the most potent growth factor for enhancing the development of human IVF-derived embryos to blastocysts (71%) and subsequent zona-hatching (82%) (Martin *et al.,* 1998). Thus, cumulative evidence suggests that this growth factor plays a major role in preimplantation embryo development and implantation in a paracrine and/or juxtacrine manner.

Cytokines

Various cytokines are considered to be involved in various aspects of implantation. Among these cytokines, IL-1, IL-6, TNF-α, and CSF-1 have been extensively studied (reviewed in Carson *et al.,* 2000; Stewart and Cullinan, 1997). However, genetic studies with mice lacking CSF-1, IL-1 or IL-6 indicate that although these factors can affect the frequency of successful implantation (Poli *et al.,* 1994; Pollard *et al.,* 1991; Zheng *et al.,* 1995), unlike LIF they are not essential for implantation (Stewart *et al.,* 1992). LIF is a member of the cytokine family that often possesses overlapping functions. This family include IL-6, IL-11, CNTF, oncostatin M, and cardiotrophin-1 (reviewed in Kishimoto *et al.,* 1994). In the mouse, LIF exists in both soluble and matrix-associated forms (Kishimoto *et al.,* 1994; Rathjen *et al.,* 1990). LIF functions through heterodimerization of LIF receptor (LIF-R) and gp 130 (Davis *et al.,* 1993).

LIF was shown to be transiently expressed in uterine glands on day 4 of pregnancy in the mouse, suggesting its role in implantation (Bhatt *et al.,* 1991). Indeed, implantation failures in LIF deficient mice confirm its essentiality in implantation (Stewart *et al.,* 1992). However, our recent studies show that *LIF* expression is biphasic in the mouse uterus on day 4 of pregnancy. Not only is *LIF* expressed in the uterine glands, but it is also expressed in stromal cells surrounding the blastocyst at the time of the attachment reaction (Song *et al.,* 2000). This suggests that LIF has dual roles: first in the preparation of the uterus and later in the attachment reaction. Reciprocal embryo transfer experiments suggest that maternal LIF is essential for implantation (Stewart *et al.,* 1992), although a role for this cytokine in embryonic functions cannot be ignored since preimplantation embryos express LIF-R and gp130, and exogenous LIF improves embryos viability and hatching (Nichols *et al.,* 1996; Robertson *et al.,* 1991). Taken together, these data suggest that both the preimplantation embryo and the uterus are potential sites of LIF action. However, embryos lacking either LIF-R or gp130 develop to the blastocyst stage and implant normally, although disruption of these genes leads to embryonic and perinatal lethality (Ware *et al.,* 1995; Yoshida *et al.,* 1996). Uterine LIF also appears to be important for implantation in other species including humans (Arici *et al.,* 1995). Indeed, LIF deficiency is apparent in infertile women and in those with unexplained recurrent abortions (Hambartsoumian, 1998). It appears that LIF expression in the uterus is maximal around the time of implantation in most species examined, although the steroid hormonal requirements for the preparation of uterine receptivity and implantation are different in different species.

Prostaglandins

It has long been speculated that prostaglandins (PGs) are involved in many aspects of the reproductive functions including implantation and decidualization. Cyclooxygenase (COX) is the rate-limiting enzyme in PG biosynthesis and exists in two isoforms, COX-1 and COX-2 (reviewed in Lim and Dey, 2000; Lim *et al.,* 1997; Lim *et al.,* 1999a; Paria *et al.,* 2000). The expression of COX-1 is constitutive, whereas that of COX-2 is inducible (Smith and deWitt, 1996). *COX* genes are expressed in a temporal and cell-specific manner in the uterus during the periimplantation period. Expression of *COX-2*, but not *COX-1*, in the luminal epithelium and stroma surrounding the blastocyst at the time of the attachment reaction suggests its role in implantation (Chakraborty *et al.,* 1996). Indeed, gene deletion studies have clearly demonstrated that COX-2 derived PGs are essential for implantation and decidualization. The implantation and decidualization defects in *COX-2(-/-)* mice are not due to any aberrant expression of other implantation-specific genes or reduced responsiveness of the uterus to steroid hormones (Lim *et al.,* 1997). Among various PGs, the levels of prostacyclin (PGI_2) are highest at the implantation sites of wild-type mice and implantation defects are partially restored in *COX-2(-/-)* mice by administration of a more stable prostacyclin agonist, carbaprostacyclin (Lim *et al.,* 1999a).

PGs can act via dual receptor signaling systems. Receptors for PGE_2, $PGF_2\alpha$, PGD_2, PGI_2 and thromboxanes have been identified as EP1-EP4, FP, DP, IP and TP, respectively, and they belong to G protein coupled cell surface receptors (reviewed in Lim and

Dey, 2000; Negishi *et al.,* 1995). Although EP receptors are expressed in the periimplantation mouse uterus, gene targeting experiments have demonstrated that three of the four EP receptor subtypes (EP_1-EP_3) are not critical for implantation, while EP_4 deficiency results in embryonic lethality and thus its role in implantation has not yet been determined (reviewed in Lim and Dey, 2000). Furthermore, mice deficient in FP or IP show normal implantation. PGs can also exert their effects by utilizing peroxisome proliferator-activated receptors (PPARs) which belong to a nuclear hormone receptor superfamily. Three members of the PPAR family are PPARα, PPARγ and PPARδ. PPARs can respond to a wide variety of ligands including natural and synthetic eicosanoids, fatty acids, hypolipidemic and hyperlipidemic drugs, and PGI_2 agonists. To act as a transcriptional activator, PPARs must form heterodimer with a member of the retinoid X receptor (RXR) subfamily (reviewed in Lim and Dey, 2000; Lim *et al.,* 1999a). We have recently shown that COX-2 derived PGI_2 participates in implantation via activation of PPARδ (Lim *et al.,* 1999a). This is consistent with reversal of implantation defects in *COX-2(-/-)* mice by a PGI_2 agonist or a PPARδ agonist (Lim *et al.,* 1999a). Taken together, the results provide evidence that COX-2-PGI_2-PPARδ signaling is important for implantation.

Concluding remarks

Although considerable information regarding the roles of growth factors, cytokines, homeotic genes and lipid mediators in embryo-uterine interactions during implantation has been generated, their hierarchical arrangement in directing uterine and embryonic functions during implantation is not clearly understood. Dysregulation of expression of *EGF-like growth factors* and *COX-2* in the uterus at the site of blastocyst apposition in LIF deficient mice with implantation failure (Song *et al.,* 2000) suggests an interaction between these molecules in this process (Fig. 2). Although *COX-2* expression is aberrant in LIF deficient uteri, uterine LIF expression is normal in *COX-2(-/-)* mice, suggesting that LIF functions upstream of COX-2 in implantation (Lim *et al.,* 1997). In contrast, LIF expression is normal in Hoxa-10 deficient uteri and vice versa (Benson *et al.,* 1996; Song *et al.,* 2000). This suggests that regulation of these two genes is independent of each other. However, uterine *COX-2* expression is attenuated during decidualization in *Hoxa-10(-/-)* mice (Lim *et al.,* 1999b). Thus, it is possible that the information sequentially originating from LIF, EGF like growth factors and Hoxa-10 signaling pathways finally converge to the COX-2 pathway for implantation (Fig. 3). Alternatively, each pathway may have its own distinctive functions or may work in parallel with each other. Thus, disruption of one pathway may result in implantation failure, and apparent dysregulation of other pathways could be the consequence of implantation failure. Finally, two of the three pathways may be interwoven and interruption of either pathway will lead to implantation failure.

Cellular and physiological aspects of preimplantation and implantation biology that had been pioneered through the years have provided the basis of our current molecular and genetic understanding of the subject. To further advance our knowledge with respect to the precise hierarchical arrangements for the functions of the genes in implantation, uterine-specific conditional gene deletion and DNA microarray approaches are of paramount importance.

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References

ARICI, A., ENGIN, O., ATTAR, E. and OLIVE, D.L. (1995). Modulation of leukemia inhibitory factor gene expression and protein synthesis in human endometrium. *J. Clin. Endocrinol. Metab.* 80: 1908-1915.

ARTLEY, J.K., BRAUDE, P.R. and JOHNSON, M.H. (1992). Gene activity and cleavage arrest in human pre-embryos. *Hum. Reprod.* 7: 1014-1021.

BAKER, J., HARDY, M.P., ZHOU, J., BONDY, C., LUPU, F., BELLVE, A.R. and EFSTRATIADIS, A. (1996). Effects of an *IGF-I* gene null mutation on mouse reproduction. *Mol. Endocrinol.* 10: 903-918.

BENSON, G.V., LIM, H., PARIA, B.C., SATOKATA, I., DEY, S.K. and MASS, R.L. (1996). Mechanisms of reduced fertility in *Hoxa-10* mutant mice: uterine homeosis and loss of maternal *Hoxa-10* expression. *Development* 122: 2687-2696.

BHATT, H., BRUNET, L.J. and STEWART, C.L. (1991). Uterine expression of leukemia inhibitory factor coincides with the onset of blastocyst implantation. *Proc. Natl. Acad. Sci. USA* 88: 11408-11412.

BINDON, B.M. (1969). The role of the pituitary gland in implantation in the mouse:delay of implantation by hypophysectomy and neurodepressive drugs. *J. Endocrinol.* 43: 225-235.

BONVENTRE, J.V., HUANG, Z., TAHERI, M.R., O'LEARY, E., LI, E., MOSKOWITZ, M.A. and SAPIRSTEIN, A. (1997). Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A_2. *Nature* 390: 622-625.

BOWMAN, P. and MCLAREN, A. (1970). Cleavage rate of mouse embryos *in vivo* and *in vitro*. *J. Embryol. Exptl. Morphol.* 24: 203-207.

CARSON, D.D., BAGCHI, I., DEY, S.K., ENDERS, A.C., FAZLEABAS, A.T., LESSEY, B.A. and YOSHINAGA, K. (2000). Embryo implantation. *Dev. Biol.* 223: 217-237.

CHAKRABORTY, I., DAS, S.K., WANG, J. and DEY, S.K. (1996) Developmental expression of the cyclooxygenase genes in the periimplantation mouse uterus and their differential regulation by the blastocyst and ovarian steroids. *J. Mol. Endocrinol.* 16: 107-122.

CROSS, J.C., WERB, Z. and FISHER, S.J. (1994). Implantation and the placenta: key pieces of the development puzzle. *Science* 266: 1508-1518.

DAS, S.K., WANG, X.-N., PARIA, B.C., DAMN, D., ABRAHAM, J.A., KLAGSBRUN, M., ANDREWS, G.K. and DEY, S.K. (1994). Heparin-binding EGF-like growth factor gene is induced in the mouse uterus temporally by the blastocyst solely at the site of its apposition: a possible ligand for interaction with blastocyst EGF-receptor in implantation. *Development* 120: 1071-1083.

DAS, S.K., DAS, N., WANG, J., LIM, H., SCHRYVER, B., PLOWMAN, G.D. and DEY, S.K. (1997). Expression of betacellulin and epiregulin genes in the mouse uterus temporally by the blastocyst solely at the site of its apposition is coincident with the 'window' of implantation. *Dev. Biol.* 190: 178-190.

DAVIS, S., ALDRICH, T.H., STAHL, N., PAN, L., TAGA, T., KISHIMOTO, T., IP, N.Y. and YANCOPOULOS, G.D. (1993). LIFR and gp130 as heterodimerizing signal transducers of the tripartite CNTF receptor. *Science* 260: 1805-1808.

DEANESLY, R. (1960). Implantation and early pregnancy in ovariectomized guinea-pigs. *J. Reprod. Fert.* 1: 242-248.

DEY, S.K. (1996). Implantation. In: *Reproductive Endocrinology, Surgery and Technology* (Adashi, E.Y., Rock, J.A. & Rosenwaks, Z., eds.) pp. 421-434. Lippincott-Raven Publishers, New York.

DE-ZIEGLER, D., FANCHIN, R., DE-MOUSTIER, B. and BULLETTI, C. (1998). The hormonal control of endometrial receptivity: estrogen (E_2) and progesterone. *J. Reprod. Immunol.* 39: 149-166.

ENDERS, A.C. and SCHLAFKE, S. (1967). A morphological analysis of the early implantation stages in the rat. *Am. J. Anat.* 120: 185-226.

FELDMAN, B., POUEYMIROU, W., PAPAIOANNOU, V.E., DECHIARA, T.M. and GOLDFARB, M. (1995). Requirement of FGF-4 for postimplantation mouse development. *Science* 267: 246-249.

FISHER, C.R., GRAVES, K.H., PARLOW, A.F. and SIMPSON, E.R. (1998). Characterization of mice deficient in aromatase (ArKO) due to targeted disruption of the

cyp-19 gene. *Proc. Natl. Acad. Sci. USA* 95, 6965-6970.

FRIEDRICH, G. and SORIANO, P. (1991). Promoter traps in embryonic stem cells: a genetic screen to identify and mutate developmental genes in mice. *Genes Dev.* 5: 1513-1523.

GARDNER, R.L. and PAPAIOANNOU, V.E. (1975). Differentiation in the trophectoderm and inner cell mass. In *The Early Development of Mammals* (Balls, M. and Wild, A.E., eds.), pp.107-132, Cambridge University Press, London.

GENDRON, R.L., PARADIS, H., HSIEH-LI, H.M., LEE, D.W., POTTER, S.S. and MARKOFF, E. (1997). Abnormal uterine stromal and glandular function associated with maternal reproductive defects in *Hoxa-11* null mice. *Biol. Reprod.* 56: 1097-1105.

GIDLEY-BAIRD, A.A. (1981). Endocrine control of implantation and delayed implantation in rats and mice. *J. Reprod. Fert. Suppl.* 29: 97-109.

HAMBARTSOUMIAN, E. (1998). Endometrial leukemia inhibitory factor (LIF) as a possible cause of unexplained infertility and multiple failures of implantation. *Am. J. Reprod. Immunol.* 39: 137-143

HOGAN, B., CONSTANTINI, F. and LACY E. (1994a). Manipulating the mouse embryo: A Laboratory Manual, Cold Spring Harbor Press, New York, p. 51.

HOGAN, B., CONSTANTINI, F. and LACY E. (1994b). Manipulating the mouse embryo: A Laboratory Manual, Cold Spring Harbor Press, New York, pp. 373-375.

HOVERSLAND, R.C., DEY, S.K. and JOHNSON, D.C. (1982). Aromatase activity in the rabbit blastocyst. *J. Reprod. Fert.* 66: 259-263.

HSIEH-LI, H.M., WITTE, D.P., WEINSTEIN, M., BRANFORD, W., LI, H., SMALL, K. and POTTER, S. S. (1995). *Hoxa 11* structure, extensive antisense transcription, and function in male and female fertility. *Development* 121: 1373-1385.

HUET-HUDSON, Y.M., ANDREWS, G.K. and DEY, S.K. (1989). Cell type-specific localization of c-myc protein in the mouse uterus: modulation by steroid hormones and analysis of the periimplantation period. *Endocrinology* 125: 1683-1690.

KISHIMOTO, T., TAGA, T. and AKIRA, S. (1994). Cytokines signal transduction. *Cell* 76: 253-262.

KWUN, J.K. and EMMENS, C.W. (1974). Hormonal requirement for implantation and pregnancy in the ovariectomized rabbit. *Aust. J. Biol. Sci.* 27: 275-283

LATASTE, F. (1891) Des variations de duree de la gestation chez les mammiferes et des circonstances qui determinent ces variations: theorie de la gestation retardee. *C. r. Seanc. Soc. Biol.* 3: 21-31.

LARUE, L., OHSUGI, M., HIRCHENHAIN, J. and KEMLER, R. (1994). E-cadherin null mutant embryos fail to form a trophectoderm epithelium. *Proc. Natl. Acad. Sci. USA* 91: 8263-8267.

LEACH, R.E., KHALIFA, R., RAMIREZ, N.D., DAS, S.K., WANG, J., DEY, S.K., ROMERO, R. and ARMANT, D.R. (1999). Multiple roles for heparin-binding epidermal growth factor are suggested by its cell specific expression during the human endometrial cycle and early placentation. *J. Clin. Endocrinol. Metab.* 84: 3355-3363.

LIM, H., PARIA, B.C., DAS, S.K., DINCHUK, J.E., LANGENBACH, R., TRZASKOS, J.M. and DEY, S.K. (1997). Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 91: 197-208.

LIM, H., GUPTA, R.A., MA, W., PARIA, B.C., MOLLER, D.E., MORROW, J.D., DUBOIS, R.N., TRZASKOS, J.M. and DEY, S.K. (1999a). Cyclooxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARδ. *Genes Dev.* 13: 1561-1574.

LIM, H., MA, L., MA, W., MASS, R.L. and DEY, S.K. (1999b). Hoxa-10 regulates uterine stromal cell responsiveness to progesterone during implantation and decidualization in the mouse. *Mol. Endocrinol.* 13: 1005-1017.

LIM, H. and DEY, S.K. (2000). PPARδ functions as a prostacyclin receptor in blastocyst implantation. *Trends in Endocrinol. Metab.* 11: 137-142.

LUBAHN, D.B., MOYER, J.S., GOLDING, T.S., COUSE, J.F., KORACH, K.S. and SMITHIES, O. (1993). Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proc. Natl. Acad. Sci. USA* 90: 11162-11166.

LUETTEKE, N.C., QIU, T.H., PEIFFER, R.L., OLIVER, P., SMITHIES, O. and LEE, D.C. (1993). TGF-α deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice. *Cell* 73: 263-278.

LUETTEKA, N.C., QIU, T.H., FENTON, S.E., TROYER, K.L., RIEDEL, R.F., CHANG, A. and LEE, D.C. (1999). Targeted inactivation of the EGF and amphiregulin genes reveals distinct roles for EGF receptor ligands in mouse mammary gland development. *Development* 126: 2739-2750.

LYDON, J.P., DEMAYO, F.J., FUNK, C.R., MANI, S.K., HUGHES, A.R., MONTGOMERY, JR. C.A., SHYAMALA, G., CONNEELY, O.M. and O'MALLEY, B.W. (1995). Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev.* 9: 2266-2278.

MARTIN, K.L., BARLOW, D.H. and SARGENT, I.L. (1998). Heparin-binding epidermal growth factor significantly improves human blastocyst development and hatching in serum-free medium. *Hum. Reprod.* 13: 1645-1652.

MENKE, T.M., and MCLAREN, A. (1970). Carbon dioxide production by mouse blastocysts during lactational delay of implantation or after ovariectomy. *J. Endocrinol.* 47: 287-294.

MCCORMACK, J.T. and GREENWALD, G.S. (1974). Progesterone and estradiol-17β concentrations in the peripheral plasma during pregnancy in the mouse. *J. Endocrinol.* 62: 101-107.

MCLAREN, A. (1971). Blastocysts in the mouse uterus: the effect of ovariectomy, progesterone and oestrogen. *J. Endocrinol.* 50: 515-526.

MCLAREN, A. (1973). Blastocyst activation. In *The regulation of Mammalian Reproduction* (Segal S, Crozier R, Corfman P. and Condliffe, P., eds), Chas. C. Thomas. Springfield, Illinois.

MCLAREN, A. (1982a). The embryo. In: *Reproduction in Mammals* (Austin, C. R. and Short, R. V. eds.), pp. 1-26, Cambridge University Press, Cambridge, UK.

MCLAREN, A. (1982b). Embryonic and fetal development. In *Reproduction in Mammals* (Austin, C.R. and Short, R.V., eds.), pp. 26-68, Cambridge University Press, Cambridge, UK.

MEYER, R.K., WOLF, R.C. and ARSLAN, M. (1969). Implantation and maintenance of pregnancy in progesterone treated ovariectomized monkeys (*Macaca mulatta*). *Proc. 2nd Int. Congr. Primat.* 2: 30-35.

MIETTINEN, P.J., BERGER, J.E., MENESES, J., PHUNG, Y., PEDERSEN, R.A., WERB, Z. and DERYNCK, R. (1995). Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. *Nature* 376: 337-341.

MORISHIMA, A., GRUMBACH, M.M., SIMPSON, E.R., FISHER, C. and QIN, K. (1995). Aromatase deficiency in male and female sibling caused by a novel mutation and the physiological role of estrogens. *J. Clin. Endocrinol. Metab.* 80: 3689-3698.

NEGISHI, M., SUGIMOTO, Y. and ICHIKAWA, A. (1995). Molecular mechanisms of diverse actions of prostanoid receptors. *Biochim. Phophys. Acta.* 1259: 109-120.

NICHOLS, J., DAVIDSON, D., TAGA, T., YOSHIDA, K., CHAMBERS, I. and SMITH, A. (1996). Complementary tissue specific expression of LIF and LIF-receptor mRNAs in early mouse embryogenesis. *Mech. Dev.* 57: 123-131.

ORSINI, M.W., and MEYER, R.K. (1962). Effect of varying doses of progesterone on implantation in the ovariectomized hamster. *Proc. Soc. Exp. Biol. Med.* 110: 713-715.

PARIA, B.C. and DEY, S.K. (1990). Preimplantatiom embryo development *in vitro*: Cooperative interactions among embryos and role of growth factors. *Proc. Natl. Acad. Sci. USA* 87: 4756-4760.

PARIA, B.C., HUET-HUDSON, Y. and DEY, S.K. (1993). Blastocyst's state of activity determines the "window" of implantation in the receptive mouse uterus. *Proc. Natl. Acad. Sci. USA* 90: 10159-10162.

PARIA, B.C., LIM, H., WANG, X.-N., LIEHR, J., DAS, S.K. and DEY, S.K. (1998). Coordination of differential effects of primary estrogen and catecholestrogen on two distinct targets mediates embryo implantation in the mouse. *Endocrinology* 139: 5235-5246.

PARIA, B.C., ELINIUS, K., KLAGSBRUN, M. and DEY, S.K. (1999). Heparin-binding EGF-like growth factor interacts with mouse blastocysts independently of ErbB1: a possible role for heparan sulfate proteoglycans and ErbB4 in blastocyst implantation. *Development* 126: 1997-2005.

PARIA, B.C., LIM, H., DAS, S.K., REESE, J. and DEY, S.K. (2000). Molecular signaling in uterine receptivity for implantation. *Seminars in Cell & Dev. Biol.* 11: 1-8.

PARR, B.A. and MCMAHON, A.P. (1995). Dorsalizing signal *Wnt-7a* required for normal polarity of D-V and A-P axes of mouse limb. *Nature* 374: 350-353.

PARR, E.L., TUNG, H.N. and PARR, M.B. (1987). Apoptosis as the mode of uterine epithelial cell death during implantation in mice and rats. *Biol. Reprod.* 36: 211-225.

PERRY, J.S., HEAP, R.B. and AMOROSO, E.C. (1973). Steroid hormone production

by pig blastocysts. *Nature* 245: 45-47.

POLI, V., BALENA, R., FATTORI, E., MARKATOS, A., YAMAMOTO, M., TANAKA, H., CILIBERTO, G., RODAN, G.A. and COSTANTINI, F. (1994). Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. *EMBO J.* 13: 1189-1196.

POLLARD, J.W., HUNT, J.S., WIKTOR-JEDRZEJCZAK, W. and STANLEY, E.R. (1991). A pregnancy defects in the osteopetrotic (op/op) mouse demonstrate the requirement of CSF-1 in female fertility. *Dev. Biol.* 148: 273-283.

PSYCHOYOS, A. (1973). Endocrine control of egg implantation. In: *Handbook of Physiology* (Greep, R.O., Astwood, E.G., and Geiger, S.R., eds.) pp.187-215. American Physiological Society, Washington, D.C., USA.

RAAB, G., KOVER, K., PARIA, B.C., DEY, S.K., EZZELL, R.M. and KLAGSBRUN, M. (1996). Mouse preimplantation blastocysts adhere to cells expressing the transmembrane form of heparin-binding EGF-like growth factor. *Development* 122: 637-645.

RATHJEN, P.D., TOTH, S., WILLIS, A., HEALTH. J.K. and SMITH, A.G. (1990). Differentiation inhibiting activity is produced in matrix-associated and diffusible forms that are generated by alternate promoter usage. *Cell* 62: 1105-1114.

RAVINDRANATH, N. and MOUDGAL, N.R. (1987). Use of tamoxifen, an antistrogen, in establishing a need for estrogen in early pregnancy in the bonet monkey (Macaca radiata). *J. Reprod. Fert.* 81: 327-336.

ROBB, L., LI, R., HARTLEY, L., NANDURKAR, H.H., KOENTGEN, F. and BEGLEY, C.G. (1998). Infertility in female mice lacking the receptor for interleukin 11 is due to a defective uterine response to implantation. *Nat. Med.* 4: 303-308.

ROBERTSON, S.A., LAVRANOS, T. and SEAMARK, R.F. (1991). In *Molecular and Cellular Immunobiology of the Maternal fetal-interface* (Wegmann, T.G., Gill, T.J., and Nisbet-Brown, E., eds.) pp. 42-72, Oxford University Press, Cambridge, UK.

ROBLERO, L.S. and GARAVAGNO, A.C. (1979). Effect of estradiol-17β and progesterone on oviductal transport and early development of mouse embryos. *J. Reprod. Fert.* 57: 91-95.

SCHLAFKE, S. and ENDERS, A.C. (1975). Cellular basis of interaction between trophoblast and uterus at implantation. *Biol. Reprod.* 12: 41-65.

SIBILIA, M. and WAGNER, E.F. (1995). Strain-dependent epithelial defects in mice lacking the EGF receptor. *Science* 269: 234-238.

SMITH, W.L. and DEWITT, D.L. (1996). Prostaglandin endoperoxide H synthase-1 and-2. *Adv. Immunol.* 62: 167-215.

SONG, H., LIM, H., DAS, S.K., PARIA, B.C. and DEY, S.K. (2000). Dysregulation of EGF family of growth factors and cyclooxygenase-2 in the uterus during the preattachment and attachment reactions of the blastocyst with the luminal epithelium correlates with implantation failure in LIF deficient mice. *Mol. Endocrinol.* 14: 1147-1161.

STEWART, C.L., KASPAR, P., BRUNET, L.J., BHATT, H., GADI, I., KONTGEN, F. and ABBONDANZO, S.J. (1992). Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature* 359: 76-79.

STEWART, C.L., and CULLINAN, E.B. (1997). Preimplantation development of the mammalian embryo and its regulation by growth factors. *Dev. Genet.* 21: 91-101.

STROMSTEDT, M., KEENEY, D.S., WATERMAN, M.R., PARIA, B.C. and DEY, S.K. (1996). Preimplantation mouse blastocysts fail to express CYP genes required for estrogen synthesis. *Mol. Reprod. Dev.* 43, 428-436.

SURANI, M.A.H. (1975). Hormonal regulation of proteins in the uterine secretion of ovariectomized rats and the implications of embryonic diapause. *J. Reprod. Fert.* 43, 411-417.

THREADGILL, D.W., DLUGOSZ, A.A., HANSEN, L.A., TENNEBAUM, T., LICHTI, U., YEE, D., LAMANTIA, C., MOURTON, T., HERRUP, K., HARRIS, R.C., BARNARD, J.A., YUSPA, S.H., COFFEY, R.J. and MAGNUSON, T. (1995). Targeted disruption of mouse EGF receptor: Effect of genetic background on mutant phenotype. *Science* 269, 230-234.

TORRES, M., STOYKOVA, A., HUBER, O., CHOWDHURY, K., BONALDO, P., MANSOURI, A., BUTZ, S., KEMLER, R. and GRUSS, P. (1997). An α-E-catenin gene trap mutation defines its function in preimplantation development. *Proc. Natl. Acad. Sci. USA* 94, 901-906.

UOZUMI, N., KUME, K., NAGASE, T., NAKATANI, N., ISHII, S., TASHIRO, F., KOMAGATA, Y., MAKI, K., IKUTA, K., OUCHI, Y., MIYAZAKI, J.-I. and SHIMIZU, T. (1997). Role of cytosolic phospholipase A_2 in allergic response and parturition. *Nature* 390, 618-622.

WANG, W., VAN DE WATER, T. and LUFKIN, T. (1998). Inner ear and maternal reproductive defects in mice lacking the *Hmx3* homeobox gene. *Developmen*t 125, 621-634.

WARE, C.B., HOROWITZ, M.C. ET AL. (1995). Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death. *Development* 121, 1283-1299.

WEITLAUF, H.M. (1974). Metabolic changes in the blastocyst of mice and rats during delayed implantation. *J. Reprod. Fert.* 39, 213-224.

WEITLAUF, H.M. (1978). Factors in uterine fluid that inhibit the incorporation of [^{3}H]uridine by blastocysts *in vitro*. *J. Reprod. Fert.* 53, 321-325.

YOO, H.J., BARLOW, D.H. and MARDON, H.J. (1997). Temporal and spatial regulation of expression of heparin-binding epidermal growth factor-like growth factor in the human endometrium: a possible role in blastocyst implantation. *Dev. Genet.* 21, 102-108.

YOSHIDA, K., TAGA, T., SAITO, M. ET AL. (1996). Targeted disruption of gp130, a common signal transducer for the interleukin 6 family of cytokines, leads to myocardial and hematological disorders. *Proc. Natl. Acad. Sci. USA* 93, 407-411.

YOSHINAGA, K. (1994). Endocrinology of Implantation. In: *Maternal-Fetal Endocrinology* (Tulchinsky, D., and Brian Little, A., eds.), pp. 335-349, W.B. Saunders Company, Philadelphia.

YOSHINAGA, K. and ADAMS, C.E. (1966). Delayed implantation in the spayed, progesterone treated adult mouse. *J. Reprod. Fert.* 12, 593-595.

ZHENG, H., FLETCHER, D., KOZAK, W., JIANG, M., HOFMAN, K.J., CONN, C.A., SOSZYNSKI, D., GRABIEC, C., TRUMBAUER, M.E., SHAW, A. ET AL (1995). Resistance to fever induction and impaired acute-phase response in interleukin-1 beta-deficient mice. *Immunity* 3, 9-19.