Mechanisms of Disease

Implantation and the Survival of Early Pregnancy

ERROL R. NORWITZ, M.D., PH.D., DANNY J. SCHUST, M.D., AND SUSAN J. FISHER, PH.D.

HUMAN reproduction entails a fundamental paradox: although it is critical to the survival of the species, the process is relatively inefficient. Maximal fecundity (the probability of conception during one menstrual cycle) is approximately 30 percent. Only 50 to 60 percent of all conceptions advance beyond 20 weeks of gestation. Of the pregnancies that are lost, 75 percent represent a failure of implantation and are therefore not clinically recognized as pregnancies. Failed implantation is also a major limiting factor in assisted reproduction. A better understanding of the molecular mechanisms responsible for implantation and placentation may improve clinicians’ ability to treat disorders related to these processes, including infertility and early pregnancy loss.

NORMAL IMPLANTATION

Early Embryonic Development

Very few specimens exist that document the first weeks of embryonic development in humans. In some cases, information about a particular stage of development comes from a single specimen. Other crucial events, such as the initial adhesion of the blastocyst to the uterine epithelium, have never been observed. Therefore, much of our understanding of early human development is inferred from studies in animals. Given that the cellular interactions culminating in implantation and placentation vary greatly even among primates, the relevance of this information is unclear. Nevertheless, certain important steps that have been identified in implantation and placentation in animals probably apply to humans. This review emphasizes those steps for which data already exist.

Fertilization occurs in the fallopian tube within 24 to 48 hours after ovulation. The initial stages of development, from fertilized ovum (zygote) to a mass of 12 to 16 cells (morula), occur as the embryo, encased in a nonadhesive protective coating known as the zona pellucida, passes through the fallopian tube. The morula enters the uterine cavity approximately two to three days after fertilization. The appearance of a fluid-filled inner cavity within the mass of cells marks the transition from morula to blastocyst and is accompanied by cellular differentiation: the surface cells become the trophoblast (and give rise to extraembryonic structures, including the placenta), and the inner cell mass gives rise to the embryo. Within 72 hours after entering the uterine cavity, the embryo hatches from the zona, thereby exposing its outer covering of syncytiotrophoblastic multinucleate trophoblasts.

Implantation occurs approximately six or seven days after conception (fertilization). Insofar as it is analogous to the events that occur in several primate species, implantation in humans probably includes three stages. The initial adhesion of the blastocyst to the uterine wall, called apposition, is unstable. Microvilli on the apical surface of syncytiotrophoblasts interdigitate with microprotrusions from the apical surface of the uterine epithelium, known as pinopodes (Fig. 1). Apposition, and consequently implantation, occurs most commonly in the upper posterior (fundal) wall of the uterus. The next stage, stable adhesion, is characterized by increased physical interaction between the blastocyst and the uterine epithelium. Shortly thereafter, invasion begins, and syncytiotrophoblasts penetrate the uterine epithelium. By then, the blastocyst is oriented with its embryonic pole toward the uterine epithelium.

By the 10th day after conception, the blastocyst is completely embedded in the stromal tissue of the uterus, the uterine epithelium has regrown to cover the site of implantation, and mononuclear cytotrophoblasts stream out of the trophoblast layer. Eventually, cytotrophoblasts invade the entire endometrium and the inner third of the myometrium (a process termed interstitial invasion), as well as the uterine vasculature (endovascular invasion). The latter process, which establishes the uteroplacental circulation, places trophoblasts in direct contact with maternal blood.

Uterine Receptivity and Blastocyst Activation

Successful implantation is the end result of complex molecular interactions between the hormonally primed uterus and a mature blastocyst (Fig. 1, 2, and 3). The
failure to synchronize the component processes involved in these interactions results in a failure of implantation.

Uterine receptivity is defined as the state during the period of endometrial maturation when the blastocyst can become implanted. Those involved in the development and use of assisted reproductive techniques for transferring embryos into the uterine cavity have identified days 20 to 24 of a regular 28-day menstrual cycle as the optimal period for implantation. The features of uterine receptivity include histologic changes (the endometrium becomes more vascular and edematous, the endometrial glands display enhanced secretory activity, and pinopodes develop on the luminal surface of the epithelium). Although these changes are useful predictors of the outcome of pregnancy, the molecular mechanisms underlying them are largely unknown.

Multiple signals synchronize the development of the blastocyst and the preparation of the uterus (Table 1). Of the many aspects of the synchronization process, the role of steroid hormones is the best understood. Implantation requires a preovulatory increase in the secretion of estradiol-17β, which stimulates the proliferation and differentiation of uterine epithelial cells. The continued production of progesterone by the corpus luteum stimulates the proliferation and differentiation of stromal cells. Downstream effectors of steroid-hormone actions include peptide hormones, growth factors, and cytokines.

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**Figure 1. Blastocyst Apposition and Adhesion.**

The diagram shows a preimplantation-stage blastocyst (approximately six to seven days after conception) and the processes thought to be necessary for uterine receptivity and blastocyst apposition and adhesion. COX-2 denotes cyclooxygenase-2, EGF epidermal growth factor, and LIF leukemia inhibiting factor.
Several factors have been identified as potential markers of endometrial receptivity. The level of leukemia inhibiting factor in both the luminal and glandular epithelium of the uterus rises dramatically in the midsecretory phase of the menstrual cycle, and diminished secretion of this factor is associated with recurrent pregnancy loss. Other molecules that are probably involved in endometrial receptivity include adhesion molecules and proteins called mucins that have high sugar content and that cause an increase in the expression of oligosaccharide receptors on the surface of endometrial epithelial cells.

The blastocyst actively participates in the process of implantation. Mechanisms that enable the blastocyst to initiate implantation (a process termed activation) include catecholestrogens, a class of estrogen metabolites. Medium in which preimplantation-stage embryos have been cultured in vitro contains many bio-

Figure 2. Blastocyst Implantation.
The diagram shows an invading blastocyst (about 9 to 10 days after conception) and the processes necessary for trophoblast invasion.
Figure 3. Maintenance of Early Pregnancy.
The diagram shows an implanted embryo (approximately 14 days after conception) and the processes necessary for the maintenance of an early pregnancy. VEGF denotes vascular endothelial growth factor, and hCG human chorionic gonadotropin.
active substances, including leukemia inhibiting factor, transforming growth factor \( \alpha \), transforming growth factor \( \beta \), platelet-derived growth factor, insulin-like growth factor II, colony-stimulating factor 1, interleukin-1, interleukin-6, prostaglandin E\(_2\), and platelet-activating factor. Evidence of signaling between the blastocyst and the uterus comes from studies in mice in which implantation has been delayed indefinitely by the manipulation of the hormones. During this delay, the expression of endometrial heparin-binding epidermal growth factor genes does not increase, even when the blastocyst is positioned next to the uterine lining. When estrogen is injected, the implantation process resumes, with the activation of the blastocyst and a rapid increase in the expression of endometrial heparin-binding epidermal growth factor genes at the site of apposition of the blastocyst.\(^{19,20}\)

Completing the loop, embryos at or near the implantation stage express epidermal-growth-factor receptors and heparan sulfate proteoglycans, both of which interact with epidermal growth factor–like ligands. The addition of heparin-binding epidermal growth factor to cultured embryos stimulates their proliferation and maturation.\(^{19,21}\) These findings are probably applicable to implantation in humans, because heparin-binding epidermal growth factor has similar effects on human embryos in vitro.\(^{22}\)

**Implantation**

The interaction between an activated blastocyst and a receptive uterus is part of a complex process that leads to implantation and the early stages of placentation development. Many of the regulatory mechanisms that have been identified govern multiple important transitions involved in this process. Thus, associating their functions with any single event draws an arbitrary distinction that does not exist in vivo. Leukemia inhibiting factor, for example, appears to be important for both decidualization and implantation.\(^{12,23}\) It is produced not only before implantation in response to estrogen in progesterone-primed uterine glands, but also at the time of implantation by stromal cells surrounding the active blastocyst.\(^{24}\)

Implantation requires the biosynthesis of prostaglandin. Cyclooxygenase (COX), the rate-limiting enzyme in the conversion of arachidonic acid to prostaglandin H\(_2\), exists in two isoforms: constitutive (COX-1) and inducible (COX-2). In the endometrium, COX-1 production decreases in response to progesterone and estradiol-17\(\beta\), and the endometrial content of COX-1 falls precipitously in the midluteal phase of the menstrual cycle in anticipation of implantation.\(^{25}\) In contrast, COX-2 production, which is not affected by steroid hormones, is restricted to the site of implantation and depends on the presence of a blastocyst that is ready to implant.\(^{25,26}\) Moreover, interleukin-1, detected in the medium in which the human embryos have been cultured,\(^{27}\) induces the expression of COX-2 genes in cultured endometrial stromal cells.\(^{28}\) Prostaglandin I\(_3\), produced by the action of COX-2 is a ligand for the nuclear receptor peroxisome-proliferator–activated receptor \( \delta \) (PPAR\( \delta \)).\(^{29}\) This interaction is probably critical, given that fetal mice lacking a related receptor (PPAR\( \gamma \)) die in the middle of the gestational period because of defective placentation.\(^{30}\)

Once implantation begins, a brief interval of stable adhesion is followed by a much longer period during which trophoblasts invade the uterus (Fig. 2). As in

### Table 1. Factors Associated with Implantation and the Maintenance of Early Pregnancy.*

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>EXAMPLES</th>
<th>SUGGESTED ROLE</th>
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<tbody>
<tr>
<td>Hormones</td>
<td>Estradiol-17(\beta), progesterone</td>
<td>Promote proliferation and differentiation of endometrial stromal and epithelial cells</td>
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<td>Changes in endometrial luminal epithelium</td>
<td>Human chorionic gonadotropin</td>
<td>Maintains progesterone release from corpus luteum</td>
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<tr>
<td>Cytokines and growth factors</td>
<td>Pinopodes; alterations in adhesion-molecule and mucin expression</td>
<td>Facilitate blastocyst capture and attachment; promote trophoblast differentiation and invasion</td>
</tr>
<tr>
<td>Immunologic factors</td>
<td>Leukemia inhibiting factor; heparin-binding epidermal growth factor; hepatocyte growth factor; interleukin; vascular endothelial growth factor</td>
<td>Facilitate signaling between blastocyst and uterus; regulate endometrial prostaglandin production; promote endometrial invasion, proliferation, and differentiation; regulate endometrial vascular permeability and remodeling</td>
</tr>
<tr>
<td>Trophoblast proteinases, inhibitors, and adhesion molecules</td>
<td>Interleukin-10; Cry (complement regulator) HLA-G</td>
<td>Immunosuppression</td>
</tr>
<tr>
<td>Other factors</td>
<td>Indoleamine 2,3-dioxygenase</td>
<td>Prevents immune recognition and rejection of fetal semi-allograft</td>
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<td></td>
<td>Matrix metalloproteinases–tissue inhibitor of metalloproteinases; cathepsin B and L; cadherins; integrins</td>
<td>Degrades tryptophan, which is essential for macrophage action</td>
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<td></td>
<td>Cyclooxygenase-2</td>
<td>Regulates prostaglandin production</td>
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<tr>
<td></td>
<td>Oxygen tension</td>
<td>Regulates the balance between trophoblast proliferation and differentiation</td>
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*This table highlights some of the more important factors and is not intended to be all-inclusive.
other biologic systems in which stable adhesion is followed by invasion, such as the extravasation of leukocytes and tumor cells, changes in the production of adhesion molecules and proteinases are implicated. The invasion of cytotrophoblasts leads to a decrease in the expression of adhesion receptors characteristic of cytotrophoblast stem cells and an increase in the expression of adhesion receptors that are characteristic of vascular cells. Besides allowing cytotrophoblasts that line maternal vessels to masquerade as vascular cells, these receptors also improve the cells’ ability to invade the uterine31,32

Invading cytotrophoblasts also increase their production of proteinase.33 For example, they increase their production and activation of matrix metalloproteinase-9, which contributes to the invasiveness of cytotrophoblasts in vitro.34 The simultaneous increase in the production of tissue inhibitor of metalloproteinase-3 provides a mechanism for restricting matrix metalloproteinase-mediated invasion.35 Matrix metalloproteinases and tissue inhibitors of metalloproteinases in maternal decidua appear to have a similar role in regulating the invasion of trophoblasts.36 Other trophoblast proteinases that may be important in invasion include cathepsin B and L.37

The molecular mechanisms that regulate the differentiation and invasion of trophoblasts are not well understood. The temporal and spatial expression of several growth factors and cytokines within the uterus (e.g., leukemia inhibiting factor,12 interleukin-1 and its receptors,38 insulin-like growth factors I and II and their binding proteins,39 colony-stimulating factor 1,40 and transforming growth factors α and β41,42 [Table 1]) suggests that they may have important functional roles. For example, interleukin-1 increases the production of matrix metalloproteinase-9 by cytotrophoblasts,43 and interleukin-1 concentrations in embryo culture medium correlate with reproductive success after in vitro fertilization.37 Decidual vascular endothelial growth factor probably promotes angiogenesis and localized vascular permeability, other key elements in implantation.44 Physiologic regulators may also be important. For example, oxygen tension promotes some aspects of trophoblast differentiation, including the production of integrin α<sub>i</sub>β<sub>i</sub>.45,46

MAINTENANCE OF EARLY PREGNANCY

Early Pregnancy Loss

The incidence of pregnancy loss after implantation is high, estimated at 25 to 40 percent.4 Although many losses involve genetic abnormalities,47 there is often no known cause. Hormonal factors, leukemia inhibiting factor, and prostaglandin pathways play important parts in successful implantation. But, given the complexities of early development, it is likely that many other mechanisms are also involved (Fig. 3 and Table 1).

Steroid Hormones

Progesterone-receptor antagonists readily induce abortion if given before seven weeks of gestation.48 Similarly, surgical removal of the corpus luteum, the source of progesterone, results in pregnancy loss.49 These data suggest that adequate progesterone production by the corpus luteum is critical to the maintenance of pregnancy until the placenta takes over this function at approximately seven to nine weeks of gestation. The corpus luteum is maintained through the continued production of chorionic gonadotropin by trophoblasts. The mode of action of progesterone is not well understood, but it appears to be partially independent of the interaction with either progesterone or glucocorticoid receptors.50 Analysis of serum hormone concentrations in pregnant women with spontaneous mutations in genes encoding steroidogenic enzymes or hormone receptors indicates that other hormones are important in this process.51 Estrogen does not have an essential role in early human pregnancy. Similarly, mineralocorticoids are not essential, and androgens are required only for sexual differentiation in the male. Whether glucocorticoids play an essential part is uncertain.

Prostaglandins

The concentrations of prostaglandins in the human decidua in early pregnancy are lower than those in the endometrium at any stage of the menstrual cycle,52,53 primarily because of a decrease in the synthesis of prostaglandins.54 Consequently, prostaglandin precursors rather than the biologically active compounds are the predominant forms in amniotic fluid and most uterine compartments. The administration of exogenous prostaglandins — intravenously, intra-amniotically, or vaginally — induces abortion in all species and at any stage of gestation. These data suggest that pregnancy is maintained by a mechanism that tonically suppresses uterine prostaglandin synthesis throughout gestation. Moreover, a defect in this inhibitory mechanism may be associated with early pregnancy loss.53,55

In sheep, the conceptus suppresses endometrial prostaglandin synthesis early in pregnancy through a mechanism that involves the production of interferon-τ. However, the same mechanism is not found in humans.56 Since endometrial prostaglandin production is also reduced in ectopic pregnancy,53 it seems likely that systemic rather than local mediators are involved. For example, progesterone decreases endometrial prostaglandin production either directly (by promoting the uptake and storage of arachidonic acid57) or indirectly (by increasing the local synthesis of endogenous inhibitors of prostaglandin synthesis, such as the secretory component of IgA44).

Regulation of Placental Growth and Differentiation

The maintenance of early pregnancy is inextricably linked with placental growth and differentiation. In
mice, the differentiation of trophoblasts is regulated by several transcription factors. Although the placentas of mice and humans differ morphologically, many of the transcriptional regulatory mechanisms may be similar.

Growth factors also function in epithelial–mesenchymal interactions that occur during early placental development. In mice that carry homozygous mutations in the scatter factor–hepatocyte growth factor gene, trophoblast differentiation is defective. Similarly, mice lacking the hepatocyte growth factor receptor (c-met) die from placental insufficiency caused by abnormal placental morphogenesis. In humans, mesenchymal cells within the stromal cores of chorionic villi produce hepatocyte growth factor, cytotrophoblasts express c-met, and hepatocyte growth factor enhances cytotrophoblast invasion.

**Immunologic Factors**

One of the most interesting functions of the placenta is the regulation of the maternal immune response such that the fetal semi-allograft is tolerated during pregnancy. Trophoblasts are presumed to be essential to this phenomenon because they lie at the maternal–fetal interface, where they are in direct contact with cells of the maternal immune system. Trophoblasts do not express classic major-histocompatibility-complex (MHC) class II molecules. Surprisingly, cytotrophoblasts express more HLA-G, a MHC class Ib molecule, as they invade the uterus. This observation, and the fact that HLA-G exhibits limited polymorphism, suggests that it has functional importance. The exact mechanisms involved are not known but may include increasing the production of inhibitory immunoglobulin-like transcript 4, an HLA-G receptor that is expressed on macrophages and a subgroup of natural killer lymphocytes.

Cytotrophoblasts that express HLA-G come in direct contact with maternal lymphocytes that are abundant in the uterus during early pregnancy. Although estimates vary, a minimum of 10 to 15 percent of all cells found in the decidua are lymphocytes. Like invasive cytotrophoblasts, these lymphocytes have unusual properties. Most are CD56+ natural killer cells. However, as compared with peripheral-blood lymphocytes, decidual leukocytes have low cytotoxic activity. Human trophoblasts help recruit these unusual maternal immune cells by means of chemokines.

Cytotoxicity against semi-allogeneic trophoblasts must be selectively inhibited. The factors responsible for this localized immunosuppression are unclear but probably include cytotrophoblast-derived interleukin-10, a cytokine that inhibits alloresponses in mixed-lymphocyte reactions. Steroid hormones, including progesterone, have similar effects. The complement system may also be involved, given that the deletion of the complement regulator Crry in mice leads to fetal loss as a result of placental inflammation.

**CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS**

**Infertility and Assisted Reproductive Technology**

Infertility may result from a failure of fertilization or from the loss of the fertilized blastocyst before implantation. The ultimate goal of understanding implantation at a molecular level is to improve the diagnosis and treatment of infertility. The most recent estimate of the likelihood of a live birth per embryo-transfer procedure with the use of standard forms of assisted reproductive technology is 27.9 percent. The failure of implantation remains a major problem and may result from diminished uterine receptivity, poor oocyte quality, or delayed implantation. The high implantation rate of donated oocytes in older women suggests that the endometrium retains normal receptivity and that oocyte quality, rather than uterine factors, determines the success of implantation. To maximize pregnancy rates after in vitro fertilization, several embryos of the two-to-eight-cell stage are transferred into the uterus, a practice that is associated with a substantial increase in higher-order multiple gestations. Although transferring fewer blastocyst-stage embryos may eliminate this problem, a better understanding of the mechanisms responsible for implantation will allow clinicians to maximize pregnancy rates while minimizing the incidence of multifetal gestations.

**Complications of Pregnancy**

At a functional level, the placenta must integrate maternal and fetal physiology, immune systems, and endocrine systems. Complications that become apparent relatively late in pregnancy may actually reflect errors that occurred much earlier in placental development.

The invasion of cytotrophoblasts to the proper depth of the uterus is a major factor in determining the outcome of pregnancy. Excessive invasion can lead to deficient development of the decidua with abnormally firm attachment of the placenta directly onto the myometrium (a condition called placenta accreta), to the extension of the placenta into the myometrium (placenta increta), or to invasion through the myometrium to the uterine serosa and even into adjacent organs (placenta percreta). Despite improvements in diagnosis and treatment, these disorders are still associated with substantial rates of maternal illness and death, primarily because of hemorrhage.
Inadequate invasion has been implicated in the pathophysiology of preeclampsia, which is the leading cause of maternal death in the industrialized world and which increases perinatal mortality by a factor of five. Although the cause of preeclampsia is unknown, the characteristic pathologic lesion is the result of shallow interstitial invasion by cytotrophoblasts and, more consistently, limited endovascular invasion.\textsuperscript{84, 85} In preeclampsia, cytotrophoblasts that invade uterine vessels fail to switch their repertoire of adhesion molecules to resemble that of vascular cells.\textsuperscript{86} Thus, the uterine arterioles remain small-bore, high-resistance vessels that cannot adequately respond to the ever-increasing fetal demands for blood flow. Determining the consequences of reduced placental perfusion and how it ultimately leads to the clinical characteristics of this syndrome remains an important challenge.\textsuperscript{87}

Normal implantation and placentation are critical for successful pregnancy. A better understanding of the molecular mechanisms responsible for these processes will improve clinicians’ ability to treat such disorders as infertility, early pregnancy loss, and preeclampsia.

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