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Hormonal regulation of implantation

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Implantation requires synchronization between the developing embryo and endometrium. The dialog between embryo and endometrium and the receptivity of the latter is under the control of the sex steroids, estrogen and progesterone, as well as other hormones, such as prolactin, calcitonin, and human chorionic gonadotropin (hCG). Although the complex process of implantation remains to be characterized fully, numerous cellular and molecular markers of endometrial receptivity—many of which are regulated hormonally—have been defined. This article addresses the endocrine-mediated aspects of implantation as they pertain to normal reproduction and assisted reproductive technology (ART).

Normal implantation

Following fertilization in the fallopian tube 24 to 48 hours after ovulation, the zygote migrates through the fallopian tube until it reaches the uterine cavity at the morula stage on Day 18 of an ideal 28-day cycle [1,2]. On Day 19, the blastocyst forms, sheds its zona pellucida, superficially apposes, and adheres to the endometrium [3]. Although the initial apposition is unstable, adhesion involves increased physical interactions between embryo and uterine epithelium [4]. This is followed by trophoblast invasion through the endometrial epithelium and underlying stroma, the inner third of the myometrium, and the uterine vasculature, all of which ultimately result in placentation [5]. Implantation occurs only during the ''window of implantation,'' which corresponds to postovulatory Days 6 to 10 in humans [6]. The endometrium is one of the

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few tissues in which implantation cannot take place except during this restricted, narrow time period [6].

In natural cycles, the implantation rate is difficult to determine because although ovulation can be confirmed, knowledge about successful fertilization and transport of the embryo to the uterine cavity is limited. The estimated rate of implantation in natural cycles—assuming the formation of only one embryo—is 15% to 30% [7]; the efficiency of human implantation is decreased compared with that of other species [8]. The implantation rate decreases with age in a nonlinear fashion until age 35, at which point there is an approximately 3% decrease per year [9].

In ART, and specifically with in vitro fertilization–embryo transfer (IVF-ET), implantation rates can be assessed more accurately. On average, the implantation rate (ie, the number of gestational sacs produced per number of healthy zygotes that are transferred into the uterine cavity) is only 10% to 15% [10,11]. Efforts to improve this rate have included allowing embryos to develop until the blastocyst stage (Day 5 versus Day 3 embryos) and using coculture techniques in which tubal, granulosa, endometrial, or other cell lines are incubated with the embryos [12].

Implicit in successful implantation is the concept of endometrial receptivity, which has been defined as "the temporally and spatially unique set of circumstances that allow for successful implantation of the embryo" [13]. Thus, a potential means of improving the implantation rate in natural and ART cycles involves the evaluation and potential manipulation of endometrial receptivity (see later discussion) which is under direct and indirect hormonal regulation.

The endometrium and the menstrual cycle

The endometrium—composed of the functionalis and basalis layers—undergoes a series of changes during each ovulatory cycle that render it temporarily amenable to implantation. The functionalis layer represents the upper two thirds of the endometrium and is the site of proliferation, secretion, and degradation, whereas the basalis layer comprises the lower one third and serves as a source for tissue regeneration. During the proliferative phase when ovarian follicular growth produces increased estrogen levels, the functionalis layer regenerates as a result of new growth of glands, stroma, and endothelial cells. Ciliogenesis—the appearance of ciliated cells around gland openings—also occurs in response to estradiol and begins on Day 7 or 8 of an ideal 28-day menstrual cycle [14]. The preovulatory increase in 17β-estradiol leads to further proliferation and differentiation of uterine epithelial cells [4].

With ovulation, the corpus luteum forms and secretes progesterone, which acts on the endometrium to promote active secretion of glycoproteins and peptides into the endometrial cavity. During this secretory phase, endometrial epithelial proliferation ceases, in part, because of progesterone-mediated blockade of es-

trogen receptor expression and stimulation of 17β-hydroxysteroid dehydrogenase and sulfotransferase activities, which metabolize the potent estradiol into estrone that is then excreted [15,16]. Approximately 7 days after the luteinizing hormone (LH) surge, peak secretory activity is reached, the endometrial stroma becomes extremely edematous, and vascular proliferation ensues in response to the sex steroids as well as local factors (eg, prostaglandins).

Decidualization, which begins late in the luteal phase under the influence of progesterone, involves increased mitosis and differentiation of stromal cells. Also associated with decidualization is the progesterone-dependent infiltration of specific leukocyte subsets into the endometrial stroma, including natural killer cells, T cells, and macrophages [17]. This steroid-mediated recruitment of leukocytes is indirect because these cells do not seem to possess estrogen or progesterone receptors [18]. In the absence of implantation, and therefore, trophoblast-derived hCG production, the transient corpus luteum undergoes regression which results in an abrupt decrease in estrogen and progesterone levels with subsequent shedding of the functionalis layer.

Mechanism of steroid hormone action

Steroid hormones act by way of their intracellular receptors to regulate gene expression of their downstream effectors, including peptide hormones, cytokines, and growth factors [4]. Unlike some steroid receptors, those for estrogen and progesterone are localized predominantly to the cell nucleus, although some nucleocytoplasmic shuttling does occur [19]. Binding of ligand to these steroid receptors leads to dimerization and subsequent binding of the steroid-receptor complexes to hormone responsive elements on DNA that results in transcriptional activation or repression of target genes [19].

Estrogen and progesterone have two receptor subtypes, α and β and A and B, respectively. Estrogen receptor (ER)-α is expressed by endometrial epithelial and stromal cells during the proliferative phase, but decreases during the secretory phase [20]. The cellular proliferation of the endometrial epithelium in response to estrogen is dependent upon stromal expression of ER-α [21]. There is little endometrial expression of ER-β; it is limited to glandular epithelial cells [22] and seems to modulate ER-α–mediated gene transcription in the uterus [23]. ER-α and -β can form homo- or heterodimers. The specific response of a cell to estrogen stimulation depends on the relative abundance of the ER subtype, the type of estrogen, and the targeted response element [19].

Similarly, the relative proportions of progesterone receptor (PR)-A and -B within a target cell determine if gene activation will occur upon hormonal stimulation because PR-A dominantly represses transcriptional activation by PR-B [24]. PR-A is expressed in the stroma and epithelium during the proliferative and secretory phases of the menstrual cycle; however, epithelial levels of PR-A gradually decrease during the secretory phase [25]. PR-B is present in glandular

and stromal nuclei only during the proliferative phase [26]. PR levels are increased by estrogens and growth factors and decrease in response to progesterone [27]. ER-β also seems to down-regulate PRs in the luminal epithelium [23]. The down-regulation of PR during the window of implantation is a prerequisite for endometrial receptivity (see later discussion) [28].

Endometrial receptivity and the luteal phase defect

Traditionally, endometrial receptivity has been assessed indirectly by the luteal phase endometrial biopsy with which a histologic determination is made regarding whether the degree of differentiation of the endometrial sample corresponds to the cycle day on which the biopsy was performed [29]. The luteal phase defect (ie, a greater than 2–3 day lag in endometrial maturation) implies a lack of endometrial receptivity. Yet, endometrial biopsies often are performed late in the luteal phase and thus, may not reflect directly on the window of implantation [13]. Furthermore, histologic endometrial maturation does not correlate necessarily with a functionally mature endometrium [30]. Recent studies suggested that two types of luteal phase defects may compromise endometrial receptivity. In the classical or type I defect, histologic endometrial maturation is delayed, whereas in the type II defect, endometrial histology is within normal limits; however, the expression of biochemical markers of maturation is impaired [31].

The type I luteal phase defect is a common condition even in fertile women; approximately one half of women who have normal cycles and who do not have diminished reproductive potential have an abnormal late luteal endometrial biopsy [32]. Furthermore, there is no statistically significant difference in the incidence of luteal phase defect between fertile and infertile women [33]. Because of the clear limitations of the endometrial biopsy and its lack of correlation with pregnancy, endometrial dating in the work-up of infertility has been discouraged [34].

The most compelling evidence for eliminating endometrial dating as part of the infertility evaluation comes from the Reproductive Medicine Network. This group reported the results of a recent large, prospective, multi-center, randomized trial at the 2002 Meeting of the American Society for Reproductive Medicine [35]. They enrolled 847 fertile and infertile women who were randomized to a mid- or late luteal endometrial biopsy. More fertile women had abnormal biopsies than did infertile women. Abnormalities were detected in 49% of fertile women and 43% of infertile women in the midluteal phase and in 35% and 23%, respectively, in the late luteal phase. These results demonstrated definitively that traditional endometrial dating is unlikely to be helpful in the most women who have infertility.

The evaluation of the endometrium for type II luteal phase defect may represent a more accurate means of assessing endometrial receptivity. Such an

evaluation would involve analysis of endometrial tissue for cellular and molecular markers that would predict successful implantation better.

Cellular/molecular markers and mechanisms underlying implantation

Implantation is a complex, hormonally-regulated process that requires synchronization between the developing embryo and differentiating endometrium. This is facilitated by molecular cross-talk between the embryo and endometrium [36]. Numerous studies have investigated potential markers of endometrial receptivity as predictors of successful implantation and, in doing so, have helped to define the cellular and molecular mechanisms by which implantation occurs. These markers include pinopodes, cell adhesion molecules, cytokines, homeobox (HOX) genes, growth factors, matrix metalloproteinases, and their inhibitors. Many clinical situations in which implantation is impaired (eg, hydrosalpinx) are associated with normal estrogen and progesterone levels; this implies that the downstream effectors of these hormones are dysregulated.

Pinopodes

With the onset of the secretory phase of the menstrual cycle, microvilli on the apical surface of the luminal endometrial epithelium fuse to form structures that are known as pinopodes [37]. The appearance of pinopodes coincides with increased progesterone levels and the down-regulation of PR-B during the window of implantation [25,38]. Although the exact function of pinopodes remains to be characterized fully, recent studies suggest that these progesterone-dependent structures extract fluid from the uterus, and thereby, facilitate closer contact between the blastocyst and endometrium [39]. The volume of uterine fluid is decreased during the window of implantation; this phenomenon is not seen following treatment with RU486, an antiprogestin [40].

Pinopodes last for only 1 or 2 days—usually Days 20 and 21 in an ideal cycle—although there is up to 5 days of variation in the timing of their appearance [37]. Furthermore, their numbers correlate with implantation [38,41]. Pinopodes form earlier in gonadotropin-stimulated cycles (Days 19–20) [42] and later in artificial, hormone replacement cycles for donor recipients (Days 21–22) [43]; this results in a loss of synchronization between the developing embryo and endometrium. Addressing this issue may represent a means of improving implantation rates in ART cycles. For example, it would be beneficial to postpone the window of implantation in women who are undergoing controlled ovarian hyperstimulation for IVF so that embryo maturation could catch up before embryo transfer [37]. Such a delay in endometrial development was accomplished in the rat with the use of the antiprogestin, RU-486, after ovulation [44].

Cell adhesion molecules

Numerous cell adhesion molecules (CAMs), including mucins [45,46] and trophinin [47], have been implicated in the attachment phase of implantation, during which they serve to tether the blastocyst to the endometrium as described by the receptor-mediated model of implantation [48]. Perhaps the best studied of the CAMs have been the integrins, which are heterodimeric glycoproteins that consist of noncovalently associated α and β subunits [49]. At least 20 types of integrin heterodimers have been defined, which form from 14 α and 9 β subunits [50]. Integrins are unusual cell surface receptors in that they bind with low affinity and are present in large numbers; this allows for ligand motility without loss of attachment.

Endometrial epithelial cells constitutively express certain integrins, whereas others are cycle-dependent [51]. Among the latter is $\alpha v\beta 3$, which is present on the apical surface of luminal endometrial cells and human embryos [52]. Osteopontin (OPN), one of the ligands for $\alpha v\beta 3$, is a glycoprotein that is secreted by the endometrium and likely serves as a bridging molecule between the embryo and endometrium [49,53]. Immunostaining for $\alpha v\beta 3$ and OPN corresponds to the endometrial pinopodes that form during the window of implantation [54].

During the secretory phase of the menstrual cycle, elevated progesterone levels increase OPN secretion [55] and result in a down-regulation of endometrial PRs [56]. The latter is associated with an increase in $\alpha v\beta 3$ expression which signals the onset of endometrial receptivity [28]. The significance of $\alpha v\beta 3$ is underscored by the finding that the loss of PR and the expression of $\alpha v\beta 3$ are delayed in infertile women who have type I luteal phase defects [28,57]. Furthermore, there is evidence that treatment of the condition that underlies the luteal phase defect or progesterone supplementation restores PR down-regulation and $\alpha v\beta 3$ expression [31,51].

Although antibodies that block $\alpha v\beta 3$ or the use of ligands that compete with OPN compromise implantation in rabbits [58], gene knock-out studies demonstrated that β_3-deficient mice are fertile. This implies that although $\alpha v\beta 3$ has a role in implantation, there is redundancy within this process [59].

Mucin 1 (MUC-1), another CAM, is a highly glycosylated glycoprotein that is present on the surface of endometrial epithelial cells, which, in response to progesterone combined with estrogen priming, is up-regulated during the window of implantation in humans [60]. Because of its extensively negatively charged nature, MUC-1 has been described as an antiadhesion molecule; it serves as such in other species where it is down-regulated during the window of implantation [61,62]. In humans, during the apposition phase of implantation, the embryo increases endometrial MUC-1 expression; this is followed by a selective decrease in MUC-1 expression, specifically at the implantation site during adhesion [63]. Thus, MUC-1 expression is regulated by steroid hormones and the implanting embryo. It was hypothesized that embryos of poor quality may not have the capacity to down-regulate MUC-1 adequately for successful implantation [63], whereas endometrial deficiency in MUC-1 may

allow for implantation of abnormal embryos that leads to recurrent pregnancy loss [64].

Cytokines

As with the CAMs, numerous cytokines have been implicated in implantation. Colony-stimulating factor (CSF)-1, for example, is expressed by human endometrium during the midproliferative and midsecretory phases [65]. Mice that have a null mutation in this gene have decreased implantation rates, which are improved with exogenous CSF-1 administration [66]. It was postulated that CSF-1 facilitates blastocyst attachment [13].

Similarly, the interleukins may facilitate the cross-talk between the embryo and endometrium. Interleukins are expressed abundantly by leukocytes that infiltrate the endometrium during progesterone-mediated decidualization [17]. Because these leukocytes do not possess steroid receptors, chemoattractant cytokines (chemokines), such as interleukin (IL)-8 and Monocyte Chemoattractan Protein-1 (MCP-1), seem to mediate the steroid-dependent recruitment of leukocytes to the endometrium [67]. Chemokines also result in the secondary induction of other cytokines, including leukemia inhibiting factor (LIF) and IL-1 and the growth factor heparin-binding epidermal growth factor (HB-EGF) [5]. IL-8 and MCP-1 are expressed by endometrial glandular and lumenal epithelial cells [68,69] where they are up-regulated by progesterone during the window of implantation [70]. This up-regulation is by way of an indirect mechanism that likely involves stromal cells or other endometrial cell types. Conversely, the embryo directly regulates endometrial IL-8 expression by increasing mRNA expression and translation, at least in vitro [70].

IL-1α, IL-1β, and the IL-1 receptor antagonist (IL-1RA) also are expressed by human endometrium [71]; levels of IL-1 receptor type 1 are maximal during the secretory phase [72]. A recent study showed that IL-1RA inhibits implantation by down-regulating the integrin subunits, α4, αv, and β3 [73]. Still, as with the integrins, there is redundancy with respect to the role that the IL-1 system plays in implantation because null mutations in the IL-1α and IL-1β genes have no appreciable effects on fertility [74].

LIF, a member of the IL-6 family, is a well-substantiated marker of implantation. This glycoprotein is expressed by human endometrium and decidua [75] where it is regulated by other cytokines and steroid hormones (eg, estrogen) [76]. There is little LIF expression in proliferative endometrium; however, levels increase during the secretory phase and reach a maximum between Days 19 and 25, which coincides with the implantation window [75]. The effects of LIF on cellular proliferation and differentiation are mediated by its receptors, LIF-R and glycoprotein 130, both of which are expressed constitutively by proliferative and secretory endometrium and trophoblasts [77]. The responsiveness of LIF-R to LIF, however, seems to be mediated by estradiol and progesterone [78]. LIF stimulates trophoblasts to increase fibronectin production, which facilitates anchoring [79] and differentiates these cells into an invasive phenotype [80].

Blastocyts cannot implant in mice that lack the LIF gene [81]. Conversely, blastocysts from LIF-deficient mice can implant into wild-type, pseudopregnant mice; this demonstrates conclusively that implantation requires maternal LIF expression [82].

That LIF is involved in human implantation is suggested by the findings that conditioned media from endometrial explants of women who have unexplained infertility have decreased levels of LIF compared with those of fertile women [83]; some infertile women have mutations in the coding region of the LIF gene [84]. Furthermore, antiprogestin treatment results in reduced LIF expression [85] and women who have unexplained infertility are more likely to have undetectable levels of LIF in their uterine flushings [86]. Similarly, women who have recurrent pregnancy loss have decreased endometrial secretion of LIF [87].

Homeobox genes

Another group of molecules that clearly are integral to implantation are the HOX genes, which encode a class of transcription factors. There are at least 39 Hox (mouse)/HOX (human) genes, all of which have a similar 183-base pair DNA sequence, the homeobox, that encodes a highly-conserved 61–amino acid domain that is known as the homeodomain [88]. Many of these transcription factors mediate embryonic development by determining regional body patterning along the anterior–posterior body axis, including that of the reproductive tract [89]. Specifically, Hoxa-9 is expressed in the developing oviduct, Hoxa-10 in the uterus, Hoxa-11 in the lower uterine segment and cervix, and Hoxa-13 in the upper vagina [90].

Unlike most Hox genes, which are expressed only during the embryonic period, those that are specific to the female reproductive tract continue to play a role in the adult [90]. For example, HOXA-10 and HOXA-11 are expressed by endometrial glands and stroma throughout the menstrual cycle [91,92]; their levels increase maximally during the midsecretory phase at the time of implantation [90].

HOXA-10 and HOXA-11 are up-regulated by 17β-estradiol and progesterone [91] and the effects of these steroids are a direct result of their receptors (ER or PR) binding to the regulatory regions of the Hoxa-10 or Hoxa-11 genes [92,93]. The continued expression of Hox/HOX genes in the female reproductive tract facilitates the growth and differentiation of the endometrium, and thereby allows for the retention of developmental plasticity, which is important for successful implantation.

One downstream target of HOXA-10 is Drosophia empty spiracles gene (EMX2) (human)/Emx2 (mouse) [94]. Emx2 is expressed in the developing brain and urogenital tract [95]; mice that lack this gene have severe urogenital malformations that result in death shortly after birth [96]. During the midluteal phase when HOXA-10 levels are maximal, EMX2 expression decline; this down-regulation occurs as a result of HOXA-10 binding to the regulatory region of the

EMX2 gene [94]. In women who have endometriosis, EMX2 expression is abnormally high during the peri-implantation period [97]; this dysregulation may be associated with the decreased implantation rates that are seen with this disease. Although the functional significance of EMX2 expression is unclear, further elucidation of the HOX system should help to define the role of EMX2 in endometrial development.

Other downstream targets for HOXA have been defined. For example, HOXA-10 binds to the β3-integrin gene and up-regulates its expression in endometrial cells; this demonstrates that HOXA-10 mediates integrin involvement in early embryo–endometrial interactions [98]. Similarly, a recent study showed that maternal Hoxa-10 expression is required for pinopode formation in the mouse [99]. Blockade of Hoxa-10 decreased pinopode number during the window of implantation in the mouse uterus, whereas overexpression of this gene increased pinopode number; this demonstrated that Hoxa-10 likely contributes to endometrial receptivity for blastocyst implantation [99]. Although there are no known human mutations in HOXA-10 or HOXA-11, women who have decreased expression of these two genes during the secretory phase have decreased implantation rates [100]. For example, endometrial HOXA-10 levels are decreased in patients who have polycystic ovarian syndrome (PCOS) [101] and in the presence of hydrosalpinx fluid [102]; the midluteal increase in HOXA-10 and HOXA-11 expression does not occur consistently in women who have endometriosis [100]. Targeted disruption of the Hoxa-10 gene in mice results in a transformation of the upper uterine segment into an oviduct-like structure and inhibits implantation, even when embryos are transferred to the grossly unaffected lower uterine segment [103,104]. Similarly, mice that have a homozygous mutation in the Hoxa-11 gene are infertile as a result of implantation defects [105] and have reduced expression of LIF [106]. Hoxa-10 and Hoxa-11 null mice produce normal numbers of embryos that are able to implant in wild-type surrogate mice, whereas wild-type embryos from surrogate mice cannot implant in the Hoxa-10 and Hoxa-11 deficient mice [103–105]. Thus, as with LIF, maternal expression of Hoxa-10 and Hoxa-11 by the endometrium is essential for implantation.

Selective alteration of endometrial Hoxa-10 expression in mice, through the use of liposome-mediated gene transfection, dramatically alters implantation, and again, demonstrates the importance of maternal Hoxa-10 for endometrial receptivity [107]. In this study, wild-type mice uteri were transfected on post-coital Day 2 with a Hoxa-10 antisense oligodeoxyribonucleotide that is designed to prevent Hoxa-10 expression. Hoxa-10 protein levels decreased as did the number of implanted embryos and the size of the resulting litters. In contrast, when the mice were transfected with Hoxa-10 cDNA, the number of implanted embryos and litter size increased significantly.

Although similar studies have not been performed in higher animal models or humans, transfection of a human endometrial adenocarcinoma cell line (Ishikawa cells) with a Hoxa-10 antisense oligodeoxyribonucleotide also resulted in decreased HOXA-10 expression. Furthermore, efficient transfection and expres-

sion of an Escherichia lacZ reporter gene was accomplished in intact human uteri ex vivo; this showed that gene transfer to the intact female reproductive tract is feasible. Thus, a gene therapy approach that involves the manipulation of HOX-10 expression may have a role in the enhancement of endometrial receptivity and implantation.

Growth factors

Growth factors are proteins that bind to specific receptors, and thereby, result in cellular differentiation or proliferation. Among the growth factors that are relevant to implantation are the HB-EGF [108,109] and amphiregulin [110]. In the mouse, HB-EGF expression is limited spatially and temporally to the site of blastocyst implantation [111], and therefore, is believed to play a role in blastocyst attachment. In women, HB-EGF also is expressed during the window of implantation [108,109], and this growth factor stimulates the growth and development of human [112] and mouse [111] blastocysts in vitro. It seems that HB-EGF also regulates endometrial $\alpha v\beta 3$ expression [113].

Like many other growth factors, endometrial HB-EGF expression is under the control of steroid hormones. For example, in the absence of estrogen, implantation in the mouse can be delayed indefinitely; however, when estrogen is provided, the blastocyst becomes activated and HB-EGF expression rapidly increases at the site of blastocyst apposition [111]. Although a role for amphiregulin in human implantation has not been defined, in the mouse, this growth factor—which is another member of the EGF family—is expressed during the period of maximal endometrial receptivity initially throughout the uterine epithelium and then, specifically at the sites of blastocyst implantation [110].

Other growth factors, such as transforming growth factor (TGF)-β, act as "maternal restraints" during implantation in that they limit trophoblast invasion [114]. TGF-β1 expression by endometrial glands and stroma increases during the secretory phase; it inhibits proliferation of cytotrophoblasts, stimulates them to differentiate into a noninvasive phenotype, and induces protease inhibitors (eg, plasminogen activator inhibitor [PAI] and tissue inhibitors of matrix metalloproteases [TIMP]-1]) that counteract extracellular matrix degradation by trophoblast-derived proteases [115].

The insulin-like growth factors (IGF)-I and -II are single-chain polypeptides that, like insulin, promote growth and differentiation of cells and also regulate cellular metabolism locally [19]. Insulin-like growth factor binding protein (IGFBP)-1, which is secreted by the secretory endometrium and decidua [116,117], serves as another restraint on trophoblast invasion by binding IGF-I and IGF-II, thereby blocking their actions. The latter growth factor is expressed in large amounts by cytotrophoblasts [117]; IGFBP-1 blocks the invasion of these cells into decidualized endometrial stromal cells in vitro [118]. The role of IGFBP-1 is not understood fully because it also was found to stimulate trophoblast invasion in other in vitro systems [119,120]. Furthermore, IGFBP-1 has been implicated in embryo recognition and the events that are associated with

early implantation because it interacts directly with integrins (eg, $\alpha5\beta1$) that are expressed by cytotrophoblasts [118,119].

Proteases and protease inhibitors

In addition to acting as a receptor for the embryo, $\alpha v\beta3$ also activates matrix metalloproteinases (MMP), such as MMP-2 [121], which degrade extracellular matrix proteins, and thereby, facilitate the invasive phase of implantation [122]. Other MMPs, including MMP-7 and MMP-11, are expressed in the endometrium during menses and the proliferative phase but are down-regulated by progesterone during the secretory phase [123]. Protease activity, and consequently, trophoblast invasion also are regulated by TIMP and other protease inhibitors, such as $\alpha2$-macroglobulin [13]. Among the TIMPs, TIMP-3 seems to be especially pertinent to implantation because it is expressed by murine decidua just adjacent to the sites of embryo implantation [124]. Furthermore, TIMP-3 also is expressed by human cytotrophoblasts [125] and decidualizing stromal cells where it is up-regulated by progesterone [126].

The invading cytotrophoblasts also express proteases (eg, MMP-9) and cathepsins B and L [127,128]. IL-1 increases MMP-9 expression by cytotrophoblasts [129]; elevated concentrations of this cytokine in embryo culture medium were correlated with successful pregnancy after IVF-ET [130].

Connexins

Connexins are a family of proteins that facilitate gap junctions between cells, and thereby, regulate cell–cell interactions. Progesterone inhibits endometrial expression of connexins, cx43 and cx26. This is believed to allow for trophoblast attachment and invasion [131].

Other endocrine mediators of implantation

Although the above discussion describes the regulation of the various markers of implantation by the sex steroids, prostaglandins and peptide hormones also play a role in implantation.

Prostaglandins

In addition to apposition, attachment, and invasion, successful implantation requires increased endometrial vascular permeability followed by angiogenesis—the generation of new blood vessels from pre-existing ones. The process of angiogenesis in the peri-implantational endometrium is not understood completely; however, it is likely that, as in other tissues, angiogenic factors (eg, vascular endothelial growth factor [VEGF]) [132] and the angiopoietins [133] are involved. Better characterized are the prostaglandins, which are arachidonic acid

metabolites that mediate a wide array of biologic processes, including angiogenesis, cellular proliferation, and differentiation. These compounds, which are generated by the cyclooxygenases (COX1 and COX2), facilitate increased vascular permeability in the endometrium during implantation [134].

Mice with null mutations in the inducible isoform of cyclooxygenase (COX2), have multifactorial reproductive failure, including impaired ovulation, fertilization, implantation, and decidualization, whereas mice that are deficient in the constitutive enzyme (COX1) are not affected in this regard. [135]. More recent studies that investigated the role of COX2 in implantation revealed that wild-type embryos are able to implant successfully in COX2-deficient mice, although there is a lag in decidualization following implantation [136].

Thus, although COX2-generated prostaglandins have a role in implantation, there, again, seems to be redundancy within this process. COX1, but not COX2 expression, is under the control of 17β-estradiol and progesterone. These steroids decrease the production of COX1, such that levels decrease drastically in the midluteal phase during the implantation window [137]. Conversely, COX2 expression is restricted to the site of implantation and is upregulated by IL-1 that is secreted by the blastocyst [129,135,137].

Calcitonin

Calcitonin is a peptide hormone that is secreted primarily by parafollicular C cells of the thyroid gland and is distributed widely throughout the body [138,139]. Although this hormone functions to decrease blood calcium by inhibiting bone osteoclast activity, it also has been implicated in the regulation of calcium flux across cell membranes [140]. Recently, calcitonin synthesis was identified in glandular epithelial cells of the rat uterus where it peaks transiently on the day before implantation [141,142]. Similarly, calcitonin is expressed by human glandular epithelial endometrium during the window of implantation where it is regulated by progesterone and inhibited by the antiprogestin, RU486 [143]. Estrogen has no direct effect on calcitonin expression, but antagonizes the effect of progesterone [142]. Administration of antisense oligodeoxynucleotides against calcitonin mRNA resulted in a significant reduction in the number of implanted embryos in the rat [144]; this implicated this peptide hormone as an important mediator of implantation. The mechanism of action may involve the dissolution of gap junctions between cells because a calcitonin-induced increase in intracellular calcium decreases endometrial cell expression of E-cadherin, a cell-surface glycoprotein that mediates cell–cell adhesion among epithelial cells [145]. Such increased permeability is hypothesized to facilitate implantation of the blastocyst [145].

Human chorionic gonadotropin

hCG, a glycoprotein hormone that is synthesized by syncytiotrophoblasts, principally serves to maintain corpus luteum progesterone function until the pla-

centa is able to take over at 60 to 70 days' gestation. The recent discovery of the chorionic gonadotropin (CG)/LH receptor in the human uterus, however, as well as LH receptor up-regulation during the period of endometrial receptivity led to much interest in the potential direct role of hCG in implantation [146]. Uterine infusion studies showed that hCG increased the secretion of several proteins from the endometrial epithelium (eg, VEGF, LIF, MMP-9), whereas it decreased IGFBP-1 and Macrophage Colony Stimulating Factor (M-CSF) expression [147]. hCG also induces the production of glycodelin, a major endometrial secretory protein that is associated with immunosuppression and epithelial cell differentiation [148]. In stromal cells, hCG promotes decidualization in the presence of estrogen and progesterone as determined by the increased transcription of prolactin, a marker of such differentiation [149].

Prolactin

Prolactin, another peptide hormone, is secreted by the endometrium during the late luteal phase and throughout pregnancy. This hormone is stimulated by progesterone and estrogen, enhances endometrial cell growth, and is requisite for implantation in mice [150]. Although the role of prolactin in human implantation is not understood fully, this hormone seems to mediate the production of macrophage activating factors (eg, interferon), and thus, may have a local immunomodulatory function [151].

Corticotropin-releasing hormone

Another hormone with a potential immunomodulatory role in implantation is corticotropin-releasing hormone (CRH), a 41–amino acid peptide that is a proinflammatory mediator and potent vasodilator. This peptide initiates the inflammatory response and stimulates leukocytes to produce IL-1 [152]. In rats, increased levels of CRH mRNA and protein have been reported at the site of implantation [153]. This peptide hormone is induced by prostaglandins and is down-regulated by estrogen and progesterone [154].

Effects of androgens on implantation

Elevated androgen levels are associated with infertility and increased miscarriage rates, in part, because of direct effects on the endometrium [155] by way of the androgen receptor, which is expressed throughout the menstrual cycle in endometrial stromal and epithelial cells [156]. Androgens seem to have pleiotropic effects on the endometrium. Although these steroids increase prolactin secretion by stromal cells in vitro [157], they negatively affect levels of glycodelin [155], a marker of endometrial secretory function. A recent study demonstrated that androstenedione inhibits endometrial cell growth and secretory activity [158]. In contrast, testosterone and dihydrotestosterone increase

endometrial concentrations of the receptor for epidermal growth factor, and thereby, promote endometrial hyperplasia, as often is seen in the setting of PCOS, a condition that is associated with hyperandrogenism [159]. In the endometrium of women who have PCOS, HOXA-10 expression is decreased markedly; similarly, testosterone decreases HOXA-10 expression in isolated endometrial cells [101]. Thus, it is not surprising that although the chronic anovulation that is associated with PCOS usually can be treated with ART, overall pregnancy rates are not high [160] and spontaneous miscarriages occur frequently [161], in part, because of the persistent effects of hyperandrogenemia on the endometrium.

Hormonal supplementation in assisted reproductive technology cycles

The increased levels of luteal phase estrogen that follow controlled ovarian hyperstimulation (COH) have a negative impact upon implantation. Such elevated levels of estrogens in the postovulatory period reflect the mechanism that is behind postcoital hormonal contraception [162]. For instance, estrogen inhibits 3β-hydroxysteroid dehydrogenase, thereby decreasing progesterone synthesis by the corpus luteum [163]. Although progesterone supplementation in animal models has been an effective means of increasing the implantation rate [164] and despite the fact that progesterone supplementation is used widely, randomized studies have not demonstrated the benefit of this practice in gonadotropin-induced cycles [165]. Conversely, in IVF-ET cycles in which prolonged GnRH analog administration is used for pituitary suppression, luteal phase serum levels of estradiol and progesterone are decreased and adversely affect implantation [166]. In this setting, luteal progesterone supplementation is beneficial [167].

Summary

Implantation is a complex, still incompletely understood process that involves the hormonally-regulated interplay between the embryo and a receptive endometrium. Although female sex steroids are the primary regulators of the cellular and molecular mediators of implantation, numerous other endocrine factors, including prostaglandins and peptide hormones, also play a role. The luteal phase endometrial biopsy is not useful for predicting endometrial receptivity, and therefore, should not be used routinely in the work-up of infertility. The analysis of cellular and molecular markers of endometrial function likely will predict successful implantation better, especially in clinical situations where estrogen and progesterone levels are within normal levels, but defects in their downstream effectors exist. Elevated androgen levels impair implantation by altering ovarian function and affecting the endometrium directly. Similarly, abnormally elevated estrogen levels in the setting of COH or postcoital contraception have detrimental effects on embryo implantation. Implanta-

tion rates in IVF-ET cycles in which GnRH agonists are used can be improved with progesterone supplementation.

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