Cyclic Decidualization of the Human Endometrium in Reproductive Health and Failure

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Decidualization denotes the transformation of endometrial stromal fibroblasts into specialized secretory decidual cells that provide a nutritive and immunoprivileged matrix essential for embryo implantation and placental development. In contrast to most mammals, decidualization of the human endometrium does not require embryo implantation. Instead, this process is driven by the postovulatory rise in progesterone levels and increasing local cAMP production. In response to falling progesterone levels, spontaneous decidualization causes menstrual shedding and cyclic regeneration of the endometrium. A growing body of evidence indicates that the shift from embryonic to maternal control of the decidual process represents a pivotal evolutionary adaptation to the challenge posed by invasive and chromosomally diverse human embryos. This concept is predicated on the ability of decidualizing stromal cells to respond to individual embryos in a manner that either promotes implantation and further development or facilitates early rejection. Furthermore, menstruation and cyclic regeneration involves stem cell recruitment and renders the endometrium intrinsically capable of adapting its decidual response to maximize reproductive success. Here we review the endocrine, paracrine, and autocrine cues that tightly govern this differentiation process.

In response to activation of various signaling pathways and genome-wide chromatin remodeling, evolutionarily conserved transcriptional factors gain access to the decidua-specific regulatory circuitry. Once initiated, the decidual process is poised to transit through distinct phenotypic phases that underpin endometrial receptivity, embryo selection, and, ultimately, resolution of pregnancy. We discuss how disorders that subvert the programming, initiation, or progression of decidualization compromise reproductive health and predispose for pregnancy failure. (Endocrine Reviews 35: 851–905, 2014)

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Abbreviations: AP-1, activating protein 1; AR, androgen receptor; BMP, bone morphogenetic protein; bZIP, basic region/leucine zipper; CD, cluster of differentiation; C/EBPβ, CCAAT/enhancer-binding protein-β; COUP-TFI, chicken ovalbumin upstream promoter-transcription factor I; COX-2, cyclooxygenase 2; CRE, CAMP response element; CREB, CRE binding protein; CREM, CRE modulator; CX43, connexin 43; CXCL, chemokine (C-X-C motif) ligand; DHEA, dehydroepiandrosterone; DKK1, dickkopf-1; DMSC, decidual MSC; EEC, endometrial epithelial cell; EPAC, exchange protein directly activated by cAMP; EPS, epithelial stem/progenitor; ERC, endometrial regenerative cell; ESR, estrogen receptor; ETS, E twenty-six; FKBP, FK506 binding protein; FOXO1, forkhead box protein O1; GADD45a, growth arrest- and DNA-damage-inducible protein of 45 kDa; GR, glucocorticoid receptor; HB-EGF, heparin-binding epidermal growth factor; HESC, human endometrial stromal cell; H3K27me3, trimethylated lysine 27 of histone 3; HOX, homeobox; HSP, heat-shock protein; HuF, human uterine fibroblast; ICER, inducible cAMP early repressor; IDO, indoleamine 2,3-dioxygenase; IFNγ, interferon-γ; IGFBP-1, IGF-binding protein-1; IFV, in vitro fertilization; JAK, Janus kinase; JNK, Jun N-terminal kinase; LEFTY, left-right determination factor; LIF, leukemia inhibitory factor; LRH-1, liver receptor homolog-1; MMP, matrix metalloproteinase; MPFA, medroxyprogesterone acetate; MR, mineralocorticoid receptor; MSC, mesenchymal stem cell; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NR3A1, nuclear receptor 3A1; PA, phosphatidic acid; PCSK6, proprotein convertase-S6; PDE, phosphodiesterase; PGDF, platelet-derived growth factor; PGE2, prostaglandin E2; PGR, progesterone receptor; PGRMC1, PGR membrane component 1; PKA, protein kinase A; PLZF, promyelocytic leukemia zinc finger; PRL, prolactin; PRL-R, PRL receptor; PTEN, PTH-like hormone; REA, repressor of estrogen receptor activity; RHL, relaxin; RPS, recurrent pregnancy loss; SENP, sentrin-specific protease; SGK1, serum- and glucocorticoid-regulated kinase 1; siRNA, small interfering RNA; SOCS, suppressor of cytokine signaling; Sp, specificity protein; STAT, signal transducers and activators of transcription; SUMO, small ubiquitin-related modifier; TSS, transcriptional start site; TWIST1, Twist-related protein 1; uDC, uterine dendritic cell; uNK, uterine natural killer; WNT, wingless-type mouse mammary tumor virus integration site.
I. Introduction

Decidualization denotes the transformation that the stromal compartment of the endometrium must undergo to accommodate pregnancy. William Potts Dewees, Professor of Obstetrics in Philadelphia in the early 19th century, was a pioneer in perinatal medicine in North America (1). In his book, *A Treatise on the Diseases of Females*, published in 1826, he describes the role of the decidua as follows: "Soon after the ovum [embryo in modern parlance; author’s comment] is deposited within the cavity of the uterus, we find it connected through the whole extent of its surface, with the internal face of this organ. Both uterus and ovum contribute to this end; on the part of the womb, we find it produces a soft spongy substance called decidua; on the part of the ovum, we discover its external covering or chorion shooting out innumerable vascular fibers — and both, when united, serve as the bond of union between ovum and uterus" (2).

The term decidua is derived from the Latin verb decidere, meaning to die, to fall off, or to detach. Decidualization of the endometrium occurs only in species in which placentation involves breaching of the luminal epithelium and invasion of maternal tissues by the trophoblast, although a decidualization-like reaction has been described in some species with noninvasive placenta, such as sheep (3). In the late 19th century, Thomas Henry Huxley, also known as Darwin’s Bulldog for his advocacy of Charles Darwin’s theory of evolution, proposed to divide the higher mammals into 2 groups, the Nondeciduata and the Deciduata. “In the Nondeciduata the fetal villi of the placenta are, at birth, simply withdrawn from the uterine fossae, into which they are received, and no part of the maternal substance is thrown off in the form of decidua, or maternal part of the placenta. In the Deciduata, on the other hand, the superficial layer of the mucous membrane of the uterus undergoes a special modification, and unites, to a greater or lesser extent, with the villi developed from the chorion of the fetus; and, at birth, this decidual and maternal part of the placenta is thrown off along with the fetus, the mucous membrane of the uterus of the parent being regenerated during, and after, each pregnancy” (4).

Within Huxley’s taxonomy of Deciduata, the process of decidual transformation varies profoundly between species. For example, decidualization can be very shallow or deep, depending on the degree of placental trophoblast invasion in a given species (5). More strikingly, decidualization of the endometrium is under maternal control in a handful of species, which includes higher primates (humans, apes, and Old World monkeys), some bats, and the elephant shrew. Maternal control means that the differentiation of endometrial stromal cells into specialist decidual cells is no longer triggered by an implanting embryo but initiated in each cycle, irrespective of the presence or absence of a conceptus. Mammals that exhibit spontaneous decidualization share a number of other reproductive characteristics, such as spontaneous ovulation, extended mating (ie, not restricted to the periovulatory period), a placenta that invades maternal blood vessels (hemochorial placentae) deeply, and give birth to only 1 or 2 well-developed offspring per pregnancy (6, 7).

However, the defining reproductive feature, exclusive to mammals exhibiting spontaneous decidualization, is menstruation (6–8). Menstruation is the periodic discharge of blood and mucosal tissue, consisting of the decidualizing superficial endometrium, triggered by a falling circulating progesterone level. The burden of abnormal menstruation and menstruation-associated disorders, such as endometriosis, in terms of health, quality of life, and socioeconomic cost, is immense. For endometriosis alone, the total annual societal cost is calculated to be approximately $65 billion in the United States (9). From an evolutionary perspective, the acquisition of maternal control over the decidual process must have been essential for reproductive fitness to justify the expenditure and cost to the mother imposed by cyclic menstruation (10).

This notion was not lost on William Potts Dewees, who wrote, “I adopt the opinion that the menstrual discharge is a genuine secretion; and that the internal face or lining of this organ is the portion which furnishes it; now it will be evident, that whenever this part is in any way deranged, its product must also be impaired; but the injury does not consist so much in the imperfect elaboration of the menstrual fluid, as in the inability of this surface to furnish a healthy decidua after impregnation has taken place; for there can be but little doubt that the same apparatus furnishes both one and the other. This condition of the uterus I have reason to think is not of frequent occurrence; an ovum may be fecundated, and duly conveyed to the cavity of the uterus; but it is suffered to perish there, from the want of a healthy decidua; it is therefore cast off unperceived, at the next menstrual purgation, and the woman is relatively barren. What strengthens this opinion is that this lesion of the uterus is frequently repaired, by either proper remedies, or by the powers of the system alone; and the woman afterwards becomes fruitful” (2).
ment and activation of mesenchymal stem cells to ensure constant tissue renewal. These powers of the system bestow extraordinary plasticity on the endometrium, enabling it to mount a response tailored to individual embryos and to adapt to reproductive failure (13). In this review, we first summarize the mechanisms that govern differentiation of endometrial stromal cells in the midluteal phase of the cycle and then expand on the role of spontaneous decidualization coupled to cyclic menstruation in embryo implantation and early pregnancy failure.

II. Decidualization: Morphological and Biochemical Features

A. Tissue changes

One of the most highly cited papers in reproduction is the classic study by Noyes, Hertig, and Rock (14), which sets out the criteria for histological dating of the endometrium. In fact, endometrial dating was pioneered in an earlier study that involved several hundred biopsies taken throughout the menstrual cycle (15). A few days after ovulation, around day 18 of the cycle, the authors observed edema in the superficial stroma, which became generalized by day 21. Beginning on day 23, they noted a marked increase in the cytoplasm of stromal cells near the terminal spiral arteries. By day 25, this process encompassed most of the superficial endometrium with the edema being replaced by contiguous large stromal cells with abundant cytoplasm and large pale nuclei. By day 27, the superficial endometrium appeared nearly solidified and the stromal cells were indistinguishable from decidual cells of pregnancy. This histological appearance of the endometrium was referred to as predecidua (15) (Figure 1).

Noyes’s criteria for histological dating are based on increased mitotic activity and pseudostratification of the nuclei in the glandular epithelium as the proliferative phase unfolds. After ovulation, this is followed by secretory transformation of the glands, which peaks in the early luteal phase. The stromal compartment also displays increased proliferative activity in the first half of the cycle, which ceases upon ovulation but recommences to some extent around days 22 to 23 when the edema regresses and the predecidual reaction begins around the terminal portion of the spiral arteries and underlying the luminal epithelium. In the late secretory phase of the cycle, the superficial layer becomes increasingly compact and massive leukocyte infiltration sets in 2 to 3 days before menstrual shedding (14).

Extensive cross-talk takes place between uterine stromal and immune cells. The uterine leukocyte population is made up largely of uterine natural killer (uNK) cells, a specialized population that stains intensely for cluster of differentiation (CD) 56 but not for CD16 antigens (CD56\textsuperscript{bright}/CD16\textsuperscript{-}) (Figure 1). After ovulation, uNK cell numbers increase dramatically, and they often make up a substantial proportion (30%–40%) of cells in the stromal compartment. The uNK cell population remains prominent in early decidua but, in the absence of pregnancy, vanishes before menses (16). In contrast to their circulating (CD56\textsuperscript{dim}/CD16\textsuperscript{-}) NK counterparts, uNK cells are not thought to function primarily as cytotoxic lymphocytes. They express killer-cell Ig-
like receptors that preferentially bind to human leukocyte antigen-C molecules expressed on placental cells, suggesting a role in maternal allorecognition of fetal trophoblast (17). There is also compelling evidence that uNK cells are important for the remodeling of spiral arteries before and during trophoblast invasion (18–20).

The uNK cells are abundant around the spiral arteries, near endometrial glands, and adjacent to extravillous trophoblast in early pregnancy. However, they are relatively sparse in the stroma underlying the luminal epithelium, and high levels of uNK cells in this region during the midluteal phase of the cycle have been associated with reproductive failure, especially recurrent pregnancy loss (21–23). The uNK cells traffic in response to chemokines such as chemokine (C-X-C motif) ligand 9 (CXCL9), CXCL10, CXCL14, and trophoblast-derived CXCL12 (19, 24). In addition, cytokines produced by resident stromal cells, including IL-11, IL-15, and IL-33, have been implicated in subsequent proliferation and maturation of uNK cells (18, 25–28). Recently, a strong inverse correlation was reported between uNK cell density in the subluminal stromal compartment and local expression of 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1), an enzyme that catalyzes the conversion of inert cortisone to active cortisol (29). The uNK cells are devoid of progesterone receptors (PGRs) but express glucocorticoid receptors (GRs), which may mediate the effects of progesterone on this cell type (30), and oral glucocorticoids markedly reduce the subluminal density of uNK cells in midluteal endometrium (31). Taken together, these observations indicate that complex and dynamic gradients of chemotactants and -repellents control the spatiotemporal distribution of uNK cells in the peri-implantation endometrium.

CD163+ macrophages rapidly accumulate in the superficial stroma after day 22 of a standardized 28-day cycle (32), and levels peak before menstruation in response to a fall in circulating progesterone (33, 34). In addition, uNK cells and macrophages, the endometrium contains scattered T cells with no apparent cycle-dependent changes, and uterine dendritic cells (uDCs), which are rare in luteal endometrium but more abundant in the decidua changes, and uterine dendritic cells (uDCs), which are rare in luteal endometrium but more abundant in the decidua

Decidualization is further characterized by profound changes in the composition of the ECM. Although collagen I, III, and VI are diffusely distributed in endometrial stroma throughout the cycle, pericellular collagen IV and laminin markedly increase surrounding decidualizing endometrial stromal cells (47). At the end of the first trimester of pregnancy, decidual cells are separated from each other by an abundant ECM and appear to be surrounded by a basement membrane. Short processes protrude through gaps in the basement membrane seemingly connecting with protrusions from the same rather than neighboring cells (39). The pericellular basement membrane of large, mature decidual cells (>25 μm) contains laminin, collagen IV, fibronectin, and heparan sulfate proteogly-
can. Large decidual cells are prominent and present throughout pregnancy. Smaller (15–25 μm) rounded cells and fibroblast-like elongated cells are also found in early pregnancy, suggesting that they represent populations at earlier stages of decidualization. Predecidual cells of the late secretory endometrium resemble intermediate-size decidual cells of pregnancy and also deposit laminin (48). Decidual cells are positive for vimentin and desmin but negative for cytokeratin and thus easily distinguishable from epithelial and trophoblast cells, which exhibit a reverse phenotype (49).

C. Decidual markers

Amniotic fluid contains an abundant amount of prolactin (PRL) with levels peaking between 18 and 26 weeks of pregnancy. The concentrations of amniotic PRL can exceed circulating levels by 50- to 100-fold (50, 51). In 1978, it was discovered that the source of amniotic PRL is not the pituitary but the decidua (52, 53). Endometrial PRL production was then shown to start before pregnancy around day 22 of the cycle in decidualizing stromal cells (54, 55). The proportion of PRL-expressing cells in the decidua increases throughout pregnancy (56), and the level of expression correlates positively with decidual cell size (57). Northern blot analysis confirmed that PRL mRNA is not expressed in either the amnion or chorion (58, 59).

Various functions have been ascribed to decidual PRL. In nonpregnant endometrium, the PRL receptor (PRL-R) localizes mainly to epithelial cells and expression peaks during the mid- to late-secretory phase (60). A paracrine target regulated by stromal cell-derived PRL is the transcription factor interferon regulatory factor 1 which co-localizes with PRL-R in the glandular compartment. IRF1 expression in the late secretory phase endometrium is up-regulated after PRL treatment (61). In pregnancy, PRL-Rs are expressed by the decidua, cytotrophoblast, syncytiotrophoblast, and amniotic epithelium and by immune cells (62, 63). PRL in the uteroplacental unit has been suggested to stimulate trophoblast growth and invasion, to promote angiogenesis, to modulate nNK cell survival, to prevent immune rejection, and to regulate water transport across the amnion toward the maternal compartment (60, 64–67). Decidual PRL in rats inhibits expression of the progesterone-catabolizing enzyme 20α-hydroxysteroid dehydrogenase (20α-HSD) and of the proinflammatory cytokine IL-6, two factors potentially detrimental to pregnancy (68). Increased 20α-HSD activity may explain the failure of progesterone supplementation to rescue pregnancy beyond midterm in PRL-R−/− mice (69, 70).

Another decidual product highly enriched in amniotic fluid is IGFBP-1 (IGFBP-1, formerly known as placental protein 12) (71). IGFBP-1 levels peak around 16 weeks of gestation, preceding those of PRL (72). Like PRL, IGFBP-1 production is induced in secretory-phase endometrial explants upon treatment with progesterone (55, 73). IGFBP-1 regulates the bioavailability of IGF-1, a function that is modified by its phosphorylation status. Although the liver produces a single highly phosphorylated isoform, decidual cells produce primarily non- or hypophosphorylated variants that are less effective in sequestering IGF-1 (74). Matrix metalloproteinase (MMP)-3 and MMP-9, produced both by the decidua and the trophoblast, cleave and inactivate decidual IGFBP-1 (75). IGFBP-1 has also been reported to stimulate trophoblast invasion (76). Deregulation of the IGF/IGFBP-1 system has been implicated in pregnancy complications like preeclampsia and intrauterine growth restriction. In early pregnancy, high IGFBP-1 levels appear beneficial, whereas elevated levels in late pregnancy are associated with obstetrical disorders (74, 77, 78).

PRL and IGFBP-1 are now established and widely used markers to assess the differentiation status of HESCs in culture. Another factor highly secreted by decidualizing cells is the NODAL-signaling pathway inhibitor left-right determination factor 2 (LEFTY2), originally designated as endometrial bleeding-associated factor (79, 80). Furthermore, many putative markers of decidualizing stromal cells in the late-secretory phase are also expressed in endometrial epithelial cells, predominantly in the proliferative phase, before their expression shifts to the stromal compartment. These include key transcription factors such as forkhead box protein O1 (FOXO1) and CCAAT/enhancer-binding protein-β (CEBPP) (81) and secretory products such as wingless-type mouse mammary tumor virus integration site family member WNT-5A (82), the inhibitor of canonical Wnt signaling, dickkopf-1 (DKK1) (83), and prokineticin-1 (84). These observations combined with the morphological changes suggest that decidualization is an example of mesenchymal-epithelial transition. A recent study provided further support for this hypothesis by demonstrating that decidualization of murine or human stromal cells is associated with down- and upregulation, respectively, of mesenchymal (eg, SNAIL) and epithelial (eg, E-cadherin) markers (85). The reverse process, epithelial-mesenchymal transition, has been implicated in the very early stages of embryo implantation (86). Thus, an ability to switch back and forth between cellular phenotypes seems to be a hallmark of the peri-implantation endometrium.

The endometrium is a relatively accessible tissue, and stromal cells can be readily isolated and cultured. Consequently, primary HESCs represent an informative model system to investigate how steroid hormones regulate di-
verse cellular functions, ranging from cell cycle progression, apoptosis, and oxidative stress responses to cellular remodeling and ECM organization. Numerous protocols have been established to induce decidualization in vitro (Table 1). Commonly used protocols involve treatment of cells with 1) estradiol and progesterone or a progestin, 2) a cAMP-inducing factor or analog, or 3) a combination of cAMP analog and progesterone or progestin. The duration of treatment varies profoundly between studies, ranging from a few hours to 10 or more days. Notwithstanding the heterogeneity in experimental design, these in vitro studies illustrate the sheer extent of cellular reprogramming upon decidualization, which underpins the acquisition of specialist functions necessary for pregnancy (Supplemental Table 1). For example, the simultaneous inhibition of tissue plasminogen activator (tPA) (87) and upregulation of tissue factor (TF), the primary initiator of coagulation (88), and plasminogen activator inhibitor 1 (PAI1) (89, 90) heighten cellular hemostasis (91), thus ensuring tissue integrity during the process of endovascular trophoblast invasion and before menstruation. Notably, decidualization is associated with altered expression of numerous ligands, receptors, signal intermediates, and downstream transcription factors, which in turn coordinate the expression of differentiation-specific transcriptional networks. In addition, several key decidual regulators are upregulated in differentiating HESCs at the protein but not mRNA level, including the tumor suppressor protein p53 (92) and the metastasis suppressor CD82 (KAI-1) (93). Decidualization is also associated with profound changes in the cytoskeleton, exemplified by a decrease in α-smooth muscle actin and by a reduction of active, phosphorylated myosin light chain-2 (94–96) (Supplemental Table 1). Consequently, a variety of factors and cellular changes have been proposed to serve as putative markers of decidualization, although few are as specific and clear-cut as PRL, IGFBP-1, or LEFTY2.

### Table 1. Deciduogenic Treatments Used for In Vitro Differentiation of HESCs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration</th>
<th>Endpoint</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial explants</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>P4 ± E2</td>
<td>6–10 d</td>
<td>PRL secretion</td>
<td>55</td>
</tr>
<tr>
<td>P4</td>
<td>2–28 d</td>
<td>PRL secretion</td>
<td>141</td>
</tr>
<tr>
<td>Primary HESC cultures</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8-Br-cAMP</td>
<td>12–24 h</td>
<td>PRL promoter induction</td>
<td>297</td>
</tr>
<tr>
<td>8-Br-cAMP</td>
<td>1–3 d</td>
<td>PRL mRNA, IGFBP-1 mRNA</td>
<td>629</td>
</tr>
<tr>
<td>8-Br-cAMP</td>
<td>2 d</td>
<td>Microarray analysis (similar results compared with P4 ± E2 + EGF for 2 d)</td>
<td>179</td>
</tr>
<tr>
<td>8-Br-cAMP</td>
<td>24–48 h</td>
<td>Microarray analysis</td>
<td>98</td>
</tr>
<tr>
<td>CRH</td>
<td>8 d</td>
<td>PRL secretion (enhanced by MPA)</td>
<td>630</td>
</tr>
<tr>
<td>db-cAMP</td>
<td>4 d</td>
<td>PRL secretion (enhanced by MPA)</td>
<td>631</td>
</tr>
<tr>
<td>FSH ± LH or hCG</td>
<td>4–6 d</td>
<td>PRL secretion</td>
<td>143</td>
</tr>
<tr>
<td>MPA</td>
<td>10–20 d</td>
<td>PRL mRNA, IGFBP-1 mRNA</td>
<td>632</td>
</tr>
<tr>
<td>MPA + 8-Br-cAMP</td>
<td>4–10 d</td>
<td>PRL secretion</td>
<td>246</td>
</tr>
<tr>
<td>MPA + E2 + activin A</td>
<td>10 d</td>
<td>PRL secretion</td>
<td>194</td>
</tr>
<tr>
<td>MPA + E2 + PGE2</td>
<td>3 d</td>
<td>PRL secretion</td>
<td>633</td>
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<tr>
<td>MPA + IGFB-1</td>
<td>28 d</td>
<td>PRL secretion</td>
<td>634</td>
</tr>
<tr>
<td>MPA + RLN</td>
<td>6 d</td>
<td>PRL secretion</td>
<td>297</td>
</tr>
<tr>
<td>MPA + RLN ± E2</td>
<td>5 d</td>
<td>PRL secretion</td>
<td>635</td>
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<td>MPA or P4</td>
<td>20 d</td>
<td>PRL secretion</td>
<td>636</td>
</tr>
<tr>
<td>P4 + cortisone + 8-Br-cAMP</td>
<td>4 d</td>
<td>PRL mRNA, IGFBP-1 mRNA</td>
<td>29</td>
</tr>
<tr>
<td>P4 + DES + EGF</td>
<td>25 d</td>
<td>PRL secretion</td>
<td>637</td>
</tr>
<tr>
<td>P4 + DHT + 8-Br-cAMP</td>
<td>4–8 d</td>
<td>PRL and IGFBP-1 secretion, PRL mRNA</td>
<td>103</td>
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<tr>
<td>P4 + E2</td>
<td>10–15 d</td>
<td>PRL secretion</td>
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<tr>
<td>P4 + E2</td>
<td>14 d</td>
<td>IGFBP-1 secretion</td>
<td>170</td>
</tr>
<tr>
<td>P4 + E2 + EGF</td>
<td>10 d</td>
<td>Microarray analysis (similar results compared with 8-Br-cAMP for 2 d)</td>
<td>179</td>
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<tr>
<td>P4 + E2 + IL-11</td>
<td>12 d</td>
<td>PRL secretion, IGFBP-1 secretion</td>
<td>639</td>
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<tr>
<td>Decidual explants</td>
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<tr>
<td>P4</td>
<td>3–10 d</td>
<td>PRL secretion</td>
<td>640</td>
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</table>

Abbreviations: DES, diethylstilbestrol; E2, estradiol; P4, progesterone.
HESCs with a decidualizing stimulus for only 3 days significantly altered the expression of 3307 genes. In this study, more genes were down- than upregulated (60% vs 40%, respectively) upon decidual transformation. These differentially expressed genes are implicated in a broad spectrum of functions, such as cell cycle regulation, cytoskeletal remodeling, angiogenesis, immune modulation, oxidative stress defense, ion and water transport, responses to steroid hormones, deposition of ECM, modulation of transcription, epigenetic patterning, posttranslational modifications, and growth factor, cytokine, and chemokine signaling, to name just a few (Supplemental Table 1). It is important to emphasize that decidual gene expression will change profoundly as the differentiation process unfolds. For example, a recent PCR array analysis of 84 inflammatory mediators identified 70 upregulated cytokines, ILs, and their receptors in cells decidualized for 2 days compared with undifferentiated control cells. By 8 days of differentiation, only 12 transcripts remained elevated, whereas 34 other mRNAs were then expressed below the level of that seen in undifferentiated cells (27). This suggests that decidual transformation is at least a biphasic process, characterized initially by an acute-phase inflammatory response, which is followed by a profound anti-inflammatory response.

Proteomic analysis of intracellular proteins differentially expressed in decidualizing stromal cells revealed a majority to be associated with cell structure and motility, followed by endocytosis/exocytosis, protein biosynthesis, DNA repair, and mitosis (106). Secretome profiling for cytokines and angiogenesis-related factors showed that decidualized cells secrete substantial levels of activin A, amphiregulin, colony-stimulating factors 2 and 3, CXCL1, IL-6, and MMP-9 (107). Garrido-Gomez et al (108) used proteomic and secretomic approaches to characterize the decidual phenotype in primary cultures treated with estradiol and progesterone. They identified 47 and 18 differentially expressed intracellular and secreted proteins, respectively. Functional annotation highlighted an abundance of proteins involved in cytoskeleton organization and ECM composition, molecular chaperones, and cytokines and chemokines.

### III. Mechanisms of Decidual Transformation

#### A. Maturation of progenitor cells

The human endometrium exhibits remarkable plasticity. Endometrial regeneration and remodeling occur after parturition, after a miscarriage, and in response to menstrual shedding. It is widely assumed that tissue repair depends on endometrial progenitor/stem cells residing in the basal layer of the endometrium (109). This hypothesis is supported by the observation that endometrial glands are monoclonal in origin, indicating that they arise from a single epithelial progenitor cell. Two stem cell populations have now been identified in the endometrium: epithelial stem/progenitor (EPS) cells and mesenchymal stem cells (MSCs). Both are clonogenic, have high proliferative potential, undergo self-renewal in vitro, can differentiate into mature progeny, and reconstitute tissue in vivo (110–115). Furthermore, bone marrow-derived cells have been shown to engraft in the endometrium of both humans and mice, albeit at low levels (116, 117). Based on these observations, it has been suggested that the endogenous population of stem/progenitor cells, rather than migratory bone marrow-derived cells, are responsible for endometrial regeneration throughout the reproductive years. This hypothesis is further supported by studies demonstrating that the endometrium is rich in cells that are highly clonogenic in in vitro assays and capable of differentiating into various mesenchymal cell lineages (110, 112).

Human endometrial MSCs are characterized by the expression of CD146 (also known as melanoma cell adhesion molecule) and platelet-derived growth factor (PDGF) receptor-β (118). Intriguingly, mature HESCs are closely related to follicular dendritic cells, which also originate from PDGF receptor-β–positive perivascular adult stem/precursor cells (119, 120). Side-population cells, defined here by the ability to exclude the DNA dye Hoechst 33342, are believed to function as stem or progenitor cells and have been isolated from various tissues including the endometrium (113, 121). Endometrial side-population cells can be induced to decidualize in vitro (122) and are capable of reconstructing human endometrium when transplanted beneath the renal capsule in an immunodeficient mouse model (123). A recent study reported that magnetic-activated cell sorting of endometrial cells with the W5C5 monoclonal antibody allows efficient isolation of a cell population enriched in endometrial MSCs (118). The W5C5 antibody was subsequently shown to bind Sushi domain containing 2 (SUSD2) (124), suggesting that this integral membrane protein may be a good marker of the stem cell niche in the endometrium. Conversely, the surface marker stage-specific embryonic antigen 1 (SSEA1) was shown to enrich for an endometrial epithelial cell population from the basalis with functional features of EPS cells (125). The precise role of EPS cells and MSCs in endometrial regeneration requires further clarification. For example, genetic fate-mapping in mice strongly indicated that postpartum re-epithelialization of the endometrium is effected by transdifferentiation of stromal MSCs (126, 127).
Menstrual blood is also an important source of MSCs, designated endometrial regenerative cells (ERCs). ERCs have a similar phenotype to endometrial and bone marrow-derived MSCs, expressing CD9, CD44, CD29, CD73, CD90, and CD105, but lack the hematopoietic markers CD34, CD45, and CD133 (128, 129). ERCs display extensive proliferative activity in vitro and are karyotypically stable and rich in proangiogenic factors. Consequently, the endometrium is increasingly viewed as an important source of adult stem cells that can be used for stem cell-based therapies in regenerative medicine (130, 131). Furthermore, MSCs isolated from the decidua of term placenta (decidual MSCs [DMSCs]) have been shown to migrate toward mammary tumors in a rat tumor model. DMSCs do not express major histocompatibility complex class II and are thus immunoprivileged and can be tolerated in allogeneic transplantation, suggesting that DMSCs could serve as vehicles for anticancer drugs (132).

Progress in understanding the endometrial stem cell system is gaining momentum. A recent study reported that the abundance of clonogenic endometrial MSCs is reduced in obese women with a history of reproductive failure (133). Hence, it seems likely that defects in the endometrial stem cell niche will impact adversely on the decidual response in early pregnancy, yet the underlying mechanisms are as yet elusive.

B. Differentiation cues and pathways

1. Endocrine cues

The ovarian steroid hormones estrogen and progesterone control uterine physiology. More specifically, the postovulatory rise in circulating progesterone, produced by luteinizing granulosa cells (134), drives the differentiation of estrogen-primed endometrium. In addition to estrogens and progesterone, circulating total testosterone, free testosterone, and androstenedione also fluctuate in a cycle-dependent manner with levels peaking at ovulation (135–137). By contrast, circulating levels of dehydroepiandrosterone (DHEA) and its sulfate ester do not change during the cycle, reflecting that the bulk of these hormones arise from the adrenals (137).

Because of local expression of enzymes involved in steroid hormone biosynthesis and metabolism, tissue concentrations of ovarian hormones do not necessarily reflect circulating levels. For example, endometrial estradiol concentrations during the proliferative phase are 5 to 8 times higher than serum levels, whereas in the secretory phase, endometrial levels are about half of those in serum (138). Conversely, tissue DHEA, androstenedione, and testosterone but not DHEA-sulfate concentrations increase 3- to 4-fold in the secretory compared with proliferative endometrium (139). Endometrial progesterone levels also increase from the proliferative to the secretory phase of the cycle, although the magnitude of this rise is modest when compared with plasma levels (139).

The morphological changes associated with decidualization are first apparent approximately 9 days after ovulation in cells surrounding the terminal spiral arteries and underlying the luminal epithelium (14). This lag period, ie, the interval between the ovulatory rise in progesterone levels and the onset of decidualization, indicates that other cues are required to initiate this differentiation process (140). This notion is supported further by in vitro experiments (Table 1). In primary HESC cultures, induction of PRL secretion requires 7 to 10 days of treatment with progesterone or a progestin, a response that can be accelerated by a host of additional factors. In explant cultures from the late-secretory endometrium, PRL production ceases after 3 to 4 days but can be restored upon addition of progesterone (141). Although these observations underscore the importance of progesterone in maintaining the decidualized status, they also indicate that this hormone is not the initiation signal for this differentiation process.

The search for purported endocrine signals that govern the onset of the decidual response has not yielded conclusive results. Some studies reported that HESCs are responsive to FSH and LH as well as human chorionic gonadotropin (hCG), yet the role of gonadotropins in initiating decidualization remains controversial (142–145). Interestingly, serum levels of relaxin (RLN), which enhances decidualization in cultured HESCs (146, 147), rise 6 to 9 days after the LH surge, although this increase is rather modest (148, 149). It is increasingly apparent that the temporal and spatial expression of local factors that increase intracellular cAMP levels in HESCs control the onset of decidualization during the midluteal phase of the cycle. These include RLN, CRH, and prostaglandin E2 (PGE2) (150–153). Inhibitory signals, such as interferon-γ (IFNγ) secreted by lymphoid aggregates in the basal endometrial layer (154, 155), are likely of equal importance in the spatiotemporal control of spontaneous decidualization of human endometrium (156).

2. Paracrine and autocrine cues

Once the decidual process is initiated, differentiating HESCs secrete a number of cytokines, growth factors, and morphogens involved in propagating this process (Figure 2). As alluded to, local immune cell populations, the endometrial vasculature, and epithelial cell compartments generate additional signal gradients essential for the transformation of the stromal compartment. For example, removal of the luminal epithelium abolishes the ability of the uterus to mount a decidual reaction in both mice and rats.
IL-11 and its receptor subunit IL-11 receptor-α are localized in the decidualizing stromal cells of the mid- to late-secretory endometrium. Moreover, in IL-11 receptor-deficient mice, embryo attachment is unimpeded and decidualization is initiated but not sustained, resulting in fetal loss on day 8 of pregnancy (161).

IL-1β is an important embryonic signal that promotes implantation and full decidualization (162, 163), foremost by upregulating αvβ3 integrin, a key implantation adhesion molecule that binds a variety of ECM ligands, including laminin, fibronectin, vitronectin, and osteopontin (164, 165). Integrin αvβ3 is also induced upon decidualization (166, 167), underscoring the importance of blastocyst signaling in eliciting a supportive post nidation environment. IL-1β upregulates cyclo-oxygenase 2 (COX-2) expression, which results in enhanced PGE2 production and cAMP accumulation (168). In addition, IL-1β enhances MMP-3 activity and facilitates morphological transformation of stromal cells by promoting ECM degradation and cytoskeletal remodeling (78). Human preimplantation embryos and placental cytotrophoblasts also secrete IL-1β (78, 169). Furthermore, exposure of decidualizing HESCs to culture medium conditioned by trophoblast acutely upregulates IL-1β transcript levels (170). It has been suggested that decidual IL-1β inhibits PRL and IGFBP-1 expression through an autocrine/paracrine mechanism (171), although this notion has been contested by others (167, 172, 173). In decidualizing HESCs, IL-1β treatment enhances CXCL1 secretion, an effect transduced through the MAPK signaling cascade (174, 175). This chemokine with mitogenic properties not only feeds back onto HESCs (176) but also promotes angiogenesis (177). Conversely, exposure of HESCs to conditioned medium from uNK cells or trophoblast markedly upregulates CXCL1 among other factors (170, 174, 178–180), illustrating how both maternal and fetal inputs contribute to the organization of the fetomaternal interface.

Leukemia inhibitory factor (LIF) belongs to the IL-6-type cytokines. This critical implantation cytokine is maximally expressed in the luminal epithelium just before implantation in mice (181, 182). LIF expression in human endometrium is restricted to the epithelial compartment during the luteal phase (158, 183), and there is evidence in both humans and mice that LIF enhances decidualization of stromal cells (184). Injection of a long-acting LIF antagonist into the mouse uterus significantly reduces the extent of decidualization at implantation sites compared with control animals (184). Although the evidence that LIF is an obligatory cytokine for implantation is overwhelming, a recent randomized placebo-controlled trial found no evidence that recombinant LIF improves pregnancy rates in women with recurrent in vitro fertilization (IVF) treatment failure (185).

### a. Interleukins

Decidualizing HESCs express IL-11 in a manner that parallels PRL and IGFBP-1 secretion (158). Furthermore, inhibition of IL-11 signaling attenuates the expression of these differentiation markers (159, 160). IL-11 and its receptor subunit IL-11 receptor-α are localized in the decidualizing stromal cells of the mid- to late-secretory endometrium. Moreover, in IL-11 receptor-deficient mice, embryo attachment is unimpeded and decidualization is initiated but not sustained, resulting in fetal loss on day 8 of pregnancy (161).

### b. TGFβ superfamily and other growth factors

Deciduogenic signals induce the expression of soluble and transmembrane heparin-binding epidermal growth factor (HB-EGF) (186, 187) as well as at least 1 of its 2 cognate receptors, human epidermal growth factor receptors 1 and 4, in HESCs (95, 188). Furthermore, inhibition of HB-EGF signaling not only attenuates PRL and IGFBP-1 production in differentiating HESCs but also sensitizes these cells to apoptosis induced by proinflammatory signals (188). In the mouse uterus, HB-EGF expression at the implantation site is induced by the blastocyst, and deletion of this growth factor impairs implantation (189, 190). When placed into the receptive mouse uterus, beads soaked with HB-EGF elicit many implantation responses, including a decidual reaction and enhanced production of bone morphogenetic protein 2 (BMP2) and WNT4 (191).

Members of the TGFβ superfamily, including activins, inhibins, and follistatin, play a major role in the paracrine/autocrine regulation of the decidual process (192, 193). Production of activin A, a dimer of βA subunits, is induced in HESCs in response to cAMP signaling and rises in parallel with PRL secretion. By contrast, activin A secretion remains low if HESCs are differentiated with only progestins (194). It has been shown that activin A itself promotes the expression of decidual markers in vitro, an effect inhibited by the activin-binding protein follistatin (194). Before the onset of the decidual process in vivo, inhibin and activin dimers are produced by epithelial cells, whereas activin receptors are expressed on stromal cells. Consequently, epithelial activin potentially promotes decidual transformation of neighboring stromal cells, which is then amplified in a paracrine/autocrine loop as differentiating HESCs assume activin production (195). Local activin promotes MMP-2 production by decidualizing cells as well as by the invading trophoblast (196). Besides activin, TGFβ1 is also implicated in the cross-talk between the epithelial cells and underlying stroma. In cocultures,
progesterone induces TGFβ1 secretion by endometrial epithelial cells, which then acts on stromal cells to induce PRL and integrin β3 production (197). Furthermore, TGFβ1 establishes another autocrine loop by reinforcing its own expression in stromal cells (198).

BMP2 is a morphogen in the TGFβ superfamily. It is expressed upon decidualization of HESCs and drives the production of decidual PRL and IGFBP-1 in part through the induction of WNT4 (199). Conditional knockout studies demonstrated that BMP2 as well as WNT4 are indispensable for the decidual reaction in mice (200, 201). The importance of BMP signaling is underscored further by the observation that conditional deletion of the BMP type 1 receptor, activin-like kinase 2 (ALK2), abrogates decidualization in mice and impairs differentiation of HESCs in culture (202). Apart from WNT4, BMP2 stimulates the expression of several Frizzled proteins (eg, FZD1 and FZD3–5), which serve as WNT receptors, as well as various WNT inhibitors (eg, DKK1, DKK2, and secreted Frizzled-related protein 3) (203). Interestingly, DKK1 is also induced in response to prokineticin-1 in both epithelial cells and differentiating HESCs, and knockdown of either secreted factor is sufficient to perturb the expression of decidual markers (83).

Another putative autocrine signal and TGFβ family member in decidualizing cells is the aforementioned LEFTY2, an important inhibitor of the NODAL signaling pathway (79, 204). Like other TGFβ members, LEFTY2 is expressed as a polypeptide that requires processing for its activation. Recent studies demonstrated that decidualization of the endometrial stroma coincides with increased expression of proprotein convertase-5/6 (PC5/
Interestingly, coimmunoprecipitation experiments indicated that Notch binds PGR independently of ligand in HuFs (215), although how this interaction affects the transcriptional activity of this nuclear receptor is not yet known.

d. Lipid signaling. Apart from PGE2, lipid signaling involving substances like endocannabinoids or phosphatidic acid (PA) is also involved in decidualization. The endocannabinoid system plays multiple roles in female reproduction (217). The cannabinoid receptor-1 is markedly induced upon decidualization (97). In the presence of cannabinoid receptor-1 agonist, the decidual response is blunted, indicating cannabinoids play a role in the spatiotemporal control of this differentiation process (218). The lipid messenger PA is a product of phospholipase D1, which hydrolyzes membrane phospholipids like phosphatidylcholine. PA effects a wide range of cellular processes including cytoskeletal organization, migration, nutrient sensing, proliferation and survival (219). Phospholipase D1 activity increases in differentiating HESCs, and knockdown of this enzyme inhibits decidualization. By contrast, PA promotes decidualization in a paracrine/autocrine manner (220). The sphingolipid metabolic pathway is also highly activated in the uterus of pregnant mice (221). This pathway produces bioactive signaling metabolites as well as complex lipids that are used in membrane organization. Disruption of this pathway in the uterus compromises decidualization, leading to hemorrhage and embryonic demise (222).

e. Other local factors. Microarray studies have revealed that upon decidualization, several additional factors are secreted capable of modulating HESC differentiation in an autocrine or paracrine manner. For instance, cAMP signaling induces a rapid and marked increase in preprosomatostatin transcripts that encode for the neuropeptide somatostatin. The somatostatin receptor subtype 2, a G protein-coupled receptor linked to various signaling components (223), is also induced upon decidualization of HESCs (98). Another secreted factor purported to enhance the expression of decidual marker genes is the peptide ghrelin, the ligand of the GH secretagogue receptor. Ghrelin and the GH secretagogue receptor are present in epithelial and stromal cells and highest in the secretory phase (224, 225). Moreover, mechanical stretch has been shown to increase IGFBP-1 expression in differentiating HESCs in vitro. In addition, conditioned medium from cells exposed to the stretch stimulus further enhanced decidualization of HESCs, suggesting that uterine contraction waves may also play a role in propagating endometrial differentiation in vivo (226).
A number of additional local regulatory circuits has been described using HuFs, although it is as yet unclear whether these findings can be extrapolated to prepregnancy HESCs. For instance, PRL secretion from HuFs can be stimulated by hCG free α-subunit, but not hCG β-subunit (227), and by IGF-1 (228) and is inhibited by placental lipocortin I (229) as well as arachidonic acid derived from the fetal membranes (216). Furthermore, PRL limits decidualization in term decidual cells through activation of a negative feedback loop (230). Another inhibitory factor identified in HuFs is PTH-like hormone (PTHLH). Knockdown of this hormone enhances morphological differentiation and expression of decidual marker genes, indicating PTHLH also limits the degree of decidualization in a paracrine/autocrine fashion (231). In the cycling endometrium, PTHLH is present in both the glandular and stromal compartments and is more abundant in the proliferative compared with the secretory phase (232). PTHLH production in cultured HESCs is under positive control of estradiol (233), and PTHLH in turn stimulates cAMP production in HESCs (234), suggesting it may be involved in initializing decidualization.

Taken together, it is increasingly apparent that short- and long-range cytokines and morphogens are responsible for the spatiotemporal control of the decidual process. Complex patterns of positive and negative regulators can generate self-organizing patterns of activity as predicted by Turing’s reaction-diffusion model (235). In multiparous species, such as mice, these patterns of endometrial activity may hold the key to ensure proper spacing of implanting embryos. In the human uterus, the spatiotemporal control of the decidual process may ensure that implantation and, more importantly, placentation occurs near the uterine fundus, away from the cervix.

C. Regulation of decidual gene expression

1. Progesterone signaling

   a. Nuclear and membrane-bound PGRs. Progesterone acts by binding and activating PGR, one of the 48 members that make up the human superfamily of nuclear receptors. In common with other nuclear receptors, PGR has a modular structure made up of distinct functional domains that enable it to respond to ligand, to bind DNA, and to regulate transcription (236, 237). There are 2 dominant PGR isoforms, A and B, which are derived from different promoter usage in a single gene. PGR-B differs from PGR-A in that it contains an additional 164 amino acids at the amino terminus (238). A number of alternatively transcribed, translated, or spliced isoforms have been described, including PGR-C, PGR-M, or PGR-S. However, it remains unproven that these truncated PGR variants are actually expressed at physiologically relevant levels in vivo (239). Although PGR-A and -B display indistinguishable hormone- and DNA-binding affinities, their actions are remarkably divergent. Initial characterization of these isoforms depended on reporter assays driven by simple or complex progesterone response elements. Based on these assays, liganded PGR-A was reported to have very limited intrinsic transcriptional activity and to function primarily as a dominant inhibitor of PGR-B and various other steroid receptors, including the estrogen receptor (ESR) (240). Subsequently, PGR-A and -B were shown to govern distinct gene networks in progesterone-responsive cells (241). Furthermore, selective ablation in mice revealed that PGR-A is indispensable for ovarian and uterine functions. By contrast, PGR-B is critical for mammary gland development (242). Thus, although PGR-A is structurally a truncated version of the B isoform, it has acquired divergent functions, especially in the uterus.

   The unliganded PGR is assembled in a large multisubunit complex that contains various heat-shock proteins (HSPs) (eg, HSP90, HSP40, HSP70, and p23) and immunophilins (eg, FK506 binding proteins [FKBP] 4 and 5) (243–245). These chaperone proteins maintain the receptor in a conformation state that allows hormone binding and play a critical role in the dynamic shuttling of the receptor between nuclear and cytoplasmic compartments. Progesterone is lipophilic and freely passes through the cell membrane to bind PGR, which even in its unliganded state resides predominantly in the nucleus (246). Ligand binding triggers a conformational change in the receptor, resulting in dissociation from the chaperone proteins, dimerization, and binding of the receptor, directly or indirectly, to promoters of target genes. Because nuclear receptors are devoid of intrinsic enzymatic activity, induction or repression of transcription depends on recruitment of histone-modifying coregulators (236, 247, 248).

   Apart from regulating gene expression through PGR activation, progesterone also triggers rapid signaling effects in the cytoplasm. It is widely assumed that this non-genomic pathway is activated upon binding of progesterone to membrane-bound receptors (249, 250). Several candidate receptors have emerged in recent years; perhaps most prominently so the PGR membrane component 1 (PGRMC1) (251, 252) and various members of the progesterin and adipoQ receptor family (253, 254). Although expressed in the human endometrium in a cycle-dependent manner (255–258), the involvement of either PGRMC1 or progesterin and adipoQ receptors in relaying progesterone actions in the endometrium remains contentious (259, 260). PGRMC1, also known as α-2 receptor, is a cytochrome-related protein recently implicated in autophagy and clearance of damaged organelles (261, 262). It is pos-
sible that this function accounts for early-onset cystic glandular hyperplasia upon deletion of PGRMC1 in murine endometrium (252).

Unequivocal evidence indicates that the nuclear PGR can mediate some rapid nongenomic progestin responses. PGR has the unique property to directly interact with src-homology 3 (SH3) domains of the Src family of tyrosine kinases, including c-Src or FYN, and adapter proteins like growth factor receptor-bound protein 2 and CRK (263). Progestin-dependent activation of the MAPK pathway through c-Src is predominantly mediated by PGR-B, probably because this isoform preferentially shuttles between the cytoplasm and nucleus, whereas PGR-A mainly resides in the nucleus (263). An alternative model has been proposed based on interaction of PGR-B with the estrogen receptor-β (ESR2), which in turn triggers rapid MAPK and AKT activation in response to progestin signaling (264).

b. Progesterone signaling in decidualizing endometrial cells. In human endometrium, PGR-B and PGR-A display a dynamic cycle- and cell type-dependent expression profile. PGR-B peaks in both the stromal and epithelial compartments in the midproliferative phase, and then expression gradually declines. By contrast, PGR-A remains highly expressed in stromal cells throughout the menstrual cycle and in pregnancy, whereas expression in the epithelial compartment drops after ovulation (60). Thus, PGR-A is the dominant isoform in the endometrial stromal compartment (265). It is also a stronger putative transactivator of IGFBP1 and decidual PRL promoters than the PGR-B (266, 267).

The mechanisms that control the spatiotemporal expression of PGR in the endometrium are complex and not entirely understood. Although it is widely assumed that estrogen signaling, via activation of estrogen receptor α (ESR1, nuclear receptor 3A1 [NR3A1]), is essential for PGR expression (238, 268), uterine PGR continues to be expressed in ESR1-knockout (ERKO) mice, albeit at lower levels. Furthermore, the physiological responses to progesterone, including decidualization, are unimpaired (269). ESR1 and PGR immunostaining is prominent in proliferative epithelium and stroma. In the mid- to late-secretory phase of the cycle, ESR1 and PGR disappear in the epithelium, but expression is retained in the stromal compartment (270). A paracrine mode for local inactivation of estradiol in response to progestin signaling has been described in the endometrium. Stromal PGR mediates the induction of 17β-HSD type 2, which converts estradiol to inactive estrone, in endometrial epithelial cells (271). This may explain how progestosterone counteracts estrogen-dependent growth of endometrial epithelial cells in the luteal phase of the menstrual cycle. Several additional mechanisms govern the expression of PGR. For example, the activity of the PGR promoter is modulated by promoter-regulating RNAs (272). These RNAs enhance or inhibit gene expression through binding to sense or antisense noncoding transcripts that overlap target promoters (273). In addition, several microRNAs have been identified that target PGR mRNA stability and translation (274–276).

Gene ablation studies in mice have not only provided unequivocal evidence of the importance of PGR in the decidual process (242) but also the dependency of this process on PGR-dependent chaperones, for example FKBP4 (277, 278), and nuclear receptor coregulators, such as steroid receptor coactivator-2 (SRC-2) (279) (Table 2). Although ESR1 may be dispensable for decidualization in rodents, the coregulator repressor of estrogen receptor activity (REA; also known as prohibitin-2) is not. Homozygous deletion of this transcriptional ESR1 coregulator abolishes implantation and decidualization, leading to female sterility. Heterozygous mutant mice are subfertile and also have reduced litters (280). In human HESCs, REA expression decreases upon decidualization. Knockdown of REA by small interfering RNA (siRNA) sensitizes HESCs to decidualogenic signals (281). Taken together, these studies demonstrate that REA, at least partly by restraining estrogen signaling, controls the timing and the extent of decidualization, which in turn is critical for the synchronous development of the blastocyst and the receptive endometrium.

Primary HESCs express all the components of a functional PGR signaling pathway. Yet, very few genes are acutely responsive to treatment with progesterone, with or without estradiol, in undifferentiated HESCs (246, 282). As aforementioned, there is overwhelming evidence that activation of the cAMP pathway is essential for PGR to acquire transcriptional control over decidua-specific gene networks (246). Convergence of cAMP and PGR pathways occurs at multiple levels, including induction of decidua-specific transcription factors, epigenetic remodeling, and wholesale reprogramming of signaling and posttranslational modification pathways. We will discuss these different levels of regulation of the decidual process after an outline of cAMP signaling in HESCs.
of 2 regulatory (R) and 2 catalytic (C) subunits. Binding of two cAMP molecules to each R subunit leads to dissociation of the C subunits, which in turn catalyze phosphorylation of various cytoplasmic target proteins (283, 284). C subunits also phosphorylate and activate nuclear targets like cAMP response element (CRE) binding protein (CREB) or the related CRE modulator (CREM) (285, 286). CREB and CREM belong to the family of basic region/leucine zipper (bZIP) transcriptional regulators and bind as dimers to their cognate palindromic octamer response element, the CRE, in target promoters (287). Subsequent recruitment of coactivators like CREB binding protein (p300) with inherent histone acetyltransferase ac-

Table 2. Genes Critical to Implantation and Decidualization: Phenotypes in Female Knockout Mice

<table>
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<tr>
<th>Deleted gene</th>
<th>Phenotype*</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Acvr1 (Alk2)</td>
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<td>Bmp2</td>
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<td>Bmpr2</td>
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<td>Cd36</td>
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<tr>
<td>Ctnnb1</td>
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</tr>
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<td>Dedd</td>
<td>Decidualization failure</td>
<td>646</td>
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<tr>
<td>Dlgap5 (Hurp)</td>
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</tr>
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</tr>
</tbody>
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* Failure may be complete or partial

tivity modulates chromatin conformation and thus facilitates transcription initiation (288).

The discovery of the novel cAMP target, exchange protein directly activated by cAMP (EPAC), added a twist to the above paradigm of cAMP signaling (289). The 2 isoforms, EPAC1 and EPAC2, are stimulated by cAMP to exchange GDP with GTP on the rat sarcoma protooncogene family members Rap1 and Rap2, thereby effecting a multitude of cellular processes ranging from Ca²⁺ homeostasis and cell fate decisions to cytoskeletal dynamics and tissue remodeling (290).

Intracellular levels of cAMP, and of cGMP, depend not only on the rate of production but also the rate of degra-
...dation by cyclic nucleotide phosphodiesterases (PDEs) (291). PDE activity is induced in response to cAMP-dependent activation of PKA, thus establishing a negative feedback loop that balances intracellular cAMP levels (284). Other mechanisms by which cells avoid excessive cAMP signaling involves degradation of the C subunit of PKA, thus reducing target protein activation, and upregulation of the R subunit, which in turn leads to increased cAMP scavenging (284).

**b. cAMP signaling in decidualizing endometrial cells.** Adenyl cyclase activity in the endometrium is higher than in the myometrium, corpus luteum, or Fallopian tube (292). Furthermore, PGE2-stimulated adenyl cyclase activity rises from the proliferative to the secretory phase and peaks in the late-secretory phase (292). Congruently, cAMP content is higher in secretory-phase endometrial biopsies compared with proliferative phase samples (293). In primary culture, extended treatment of HESCs with estradiol and a progestin increases cAMP production and induces decidual marker genes. This induction, however, can be blocked with a PKA inhibitor, underscoring the importance of the cAMP pathway in the HESC response to ovarian hormones (82, 294, 295). Progesterone also potentiates PGE2-induced cAMP production (296). Persistent elevation of intracellular cAMP concentration is paramount for the decidualized status in HESCs (297). Unlike many other cell types, HESCs do not terminate cAMP-dependent PKA signaling but establish a feed-forward mechanism that involves selective downregulation of the regulatory PKA subunit regulatory PKA subunit. Consequently, the ratio of C to R subunits is shifted in favor of C, resulting in a sustained increased kinase activity in decidualizing HESCs (298).

PDE4 is the major cAMP-specific PDE isoform in HESCs. Its expression increases upon decidualization in response to PKA activation. Pharmacological inhibition of PDE4 increases intracellular cAMP in HESCs and synergizes with RLN in the induction of decidual markers. Intravaginal application of PDE4 inhibitors, such as rolipram, in combination with RLN has therefore been suggested for luteal phase support of the endometrium in subfertile women (299, 300).

EPAC1 and EPAC2 signaling also play a role in cAMP-dependent decidualization. Although treatment with an EPAC-selective cAMP analog alone is not sufficient to induce decidual markers, activation of this pathway enhances HESC differentiation in response to PKA activation or steroid hormone treatment. The siRNA-mediated knockdown of EPAC1 or EPAC2 blocks differentiation of HESCs (301). Apart from Rap2, calreticulin, a Ca$^{2+}$-binding storage protein and molecular chaperone in the endoplasmic reticulum, has been suggested to be a downstream target of EPAC2 (301).

CREB is abundantly expressed in HESCs irrespectively of their differentiation status. Upon PKA signaling, CREB is activated and drives transcription from CRE-containing promoters (297). In addition, HESCs express a complex pattern of CREM isoforms that arise by alternative splicing or alternative translation initiation. Depending on the presence or absence of a transactivation domain, CREMs function as transcriptional activators or repressors of cAMP-responsive genes. Moreover, inducible cAMP early repressor (ICER) is upregulated in decidualizing HESCs (302). ICER is generated from a cAMP-responsive intronic promoter in the CREM gene and contains only a functional DNA-binding domain. It thus acts as a repressor of CRE-responsive gene promoters, including its own promoter, and therefore should establish an autoregulatory negative feedback loop (303). However, ICER is persistently elevated in decidualizing HESCs, which reflects continued PKA activation and a dominance of stimulatory CREB and CREM isoforms that maintains cAMP responsiveness in this cell type (302).

### 3. Transcriptional regulation of decidual genes

**a. Transposon-mediated wiring of decidual gene networks.** Initial attempts at unraveling the transcriptional regulation of decidual genes were frustrated by the fact that neither progesterone nor cAMP signaling seemed to follow classical pathways in HESCs as outlined above. Two important discoveries were key to overcoming this hurdle, both related to the organization of the human *PRL* gene. First, it was shown that decidualizing HESCs, as well as human lymphocytes, use a different promoter in the *PRL* gene when compared with pituitary cells. This alternative decidual promoter flanks a noncoding exon (exon 1a) and is located approximately 6 kb upstream of the first exon (1b) transcribed in the pituitary. Activation of the decidual *PRL* promoter results in the addition of a 5′-untranslated exon to the *PRL* transcript but leaves the protein-coding region unaltered (Figure 3) (304–307). This observation explained why *PRL* expression in HESCs is insensitive to specifiers of pituitary *PRL* expression, such as bromocriptine, dopamine, TRH, or the transcription factor POU domain, class I, transcription factor 1 (306, 308). The second breakthrough came from the systematic mapping of cAMP-responsive sequences in the decidual *PRL* promoter. This led first to the identification of a CRE-like element in the decidual *PRL* promoter, located near the transcriptional start site (TSS) and responsible for rapid but modest transcriptional activation in response to cAMP signaling. In addition, an enhancer region was identified in the decidual *PRL* promoter and mapped to −332/−270...
bp relative to the TSS (297). This regulatory region was shown to be responsible for enhanced and sustained PRL expression, apparent after 12 hours or more of cAMP stimulation (297, 309). Critically, it also confers responsiveness of the decidual PRL promoter to PGR signaling (267).

The (−332/−270) enhancer region in the decidual PRL promoter was subsequently shown to be part of a transposable genetic element, termed MER20 (Figure 3). Transposable elements comprise a vast array of DNA sequences that can move to new sites in genomes, either by a cut-and-paste mechanism (transposons) or through an RNA intermediate (retrotransposons) (310). MER20 is a cut-and-paste DNA transposon (311), found only in placental mammals. Insertion of MER20 in the ancestral PRL locus coincided with the placenta acquiring an invasive phenotype (312–314). MER20 transposons have epigenetic signatures of enhancers, insulators, and repressors, indicating they play an important role in wiring the gene regulatory landscape in differentiating HESCs (313). In agreement, MER20 transposons are enriched near decidual genes and contain binding motifs for core decidual transcription factors, such as PGR, C/EBPβ, forkhead box transcription factors of the O subclass (FOXOs), and homeobox (HOX) proteins. MER20 transposons also contain cis-regulatory elements for E twenty-six 1 (ETS1) and a number of ubiquitous transcription regulators, such as CREB binding protein/p300 and Yin Yang 1 (313).

At a functional level, MER20s from 20 genes, including PRL, WNT4, WNT5A, INHA (inhibin α-chain), and HSD11B1, were shown to confer responsiveness to cAMP and progesterin signaling in reporter gene assays when transfected into HESCs but not other cell types. Furthermore, a screen of 34 human tissues showed that coexpression of key transcriptional initiators (eg, PGR, FOXO1, C/EBPβ, and HOXA11) is confined to the uterus (313). Taken together, these observations indicate that insertion of transposable elements such as MER20 in the genome introduced novel cis-regulatory elements (enhancer/repressor elements and promoters) that co-opted the cAMP and progesterone signaling pathways to drive expression of decidua-specific genes (313). Depending on the promoter context and state of differentiation, a host of transcription factors in addition to the core initiators are involved in fine-tuning decidual gene networks.

Endometrial PRL expression is not shared by all placental mammals, because it is not evident in rabbits, pigs, dogs, or armadillos (315). It has been demonstrated in

Figure 3. Structure of the human PRL gene and regulation of the upstream decidual promoter by convergence of progesterone and CAMP signaling. In decidual and lymphoid cells, an alternative promoter of the hPRL gene is used, which is located 5.9 kb upstream of the pituitary promoter. Transcription initiated at upstream exon 1a results in generation of a decidual/lymphoid PRL mRNA with an extended 5′-untranslated region (UTR) but a protein coding region identical to the pituitary PRL mRNA. Exon 1a is embedded within the transposon MER39: the critical cis-regulatory region −332/−270 relative to the decidual/lymphoid TSS lies within transposon MER20. In MER20, selected transcription factor binding sites are shown that were predicted by Transfac or by Promo3. Those supported by experimental evidence are marked with an asterisk.
primates, mice, and elephants and involves, as in the human, the use of an alternative upstream promoter and splicing of alternative 5′ noncoding exons (315). The human decidual PRL promoter is located within a 498-bp long terminal repeat (LTR)-type transposable element, termed MER39, which was originally thought to be primate-specific (312). Subsequent analyses revealed the presence of MER39 in the PRL locus of all primates and rodents (315, 316) (Figure 3). In the mouse, however, endometrial Prl expression is initiated within a different transposon, MER77; and in the elephant, the alternative PRL promoter is derived from the transposable element L1–2_LA (315). Apparently, adaptive evolution of endometrial PRL expression in placental mammals occurred through the recruitment of different transposable elements in the primate, rodent, and elephant lineages.

b. CCAAT/enhancer-binding proteins. As outlined above, cAMP-dependent decidual PRL activation in HESCs is a biphasic process, with the conventional PKA/CREB/CREM pathway responsible for initial modest induction and the assembly of transcriptional complexes on the MER20 enhancer for the sharp increase in expression upon prolonged cAMP stimulation. C/EBPs belong to the transcription factor superfamily of bZIP DNA-binding proteins (317). Several lines of evidence indicate that binding of C/EBPβ to the core enhancer sequence within MER20 represents a priming event that enables recruitment of other key transcription factors. First, increased expression of C/EBPs, specifically the β- and δ-isozymes, is a relatively early event in HESCs treated with a cAMP analog (318). Furthermore, the DNA binding activity of C/EBPβ increases markedly in response to cAMP signaling in HESCs, which is mediated by activation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complexes and a surge in endogenous reactive oxygen species (309). Notably, activation of the NADPH oxidase complex and transient redox signaling is not specific to decidualizing HESCs but a highly conserved differentiation signal in a variety of cells, including embryonic stem cells, vascular smooth muscle cells, and preadipocytes (319–321). Once bound to the core enhancer sequence within MER20, C/EBPβ has been shown to recruit and functionally cooperate with PGR-A as well as FOXO1 (81, 267).

In the endometrium, increased nuclear staining of C/EBPβ is observed in the stromal compartment during the midsecretory phase of the cycle (81, 322). Silencing of C/EBPβ in HESCs disturbs G1-S cell cycle transition and impairs IL-11 signaling in decidualizing cells (323). Moreover, lack of C/EBPβ diminishes expression of the morphogen BMP2, critically involved in decidualization of both mouse and human endometrial stromal cells, and of its downstream target WNT4 (323). Finally, C/EBPβ-deficient female mice are infertile due to defective decidualization (324), emphasizing the evolutionarily conserved function of this transcription factor in endometrial preparation for pregnancy.

c. FOXO transcription factors. The FOXO subfamily of forkhead transcription factors consists of 3 members in mammals, FOXO1, FOXO3a, and FOXO4. Functionally, they represent a hybrid subclass in that they act not only as transcription factors but also as pioneering factors that open up local chromatin structures and thereby increase accessibility for other transcription factors (325). FOXO proteins are downstream targets of the phosphatidylinositol 3-kinase/AKT pathway, which mediates growth factor signaling (326, 327). FOXO proteins are key regulators of cell fate decisions, capable of triggering cell cycle arrest, senescence, and apoptosis as well as cellular differentiation. The transcriptional output of FOXOs depends on site-specific posttranslational modifications, such as phosphorylation, methylation, or ubiquitination, which change dynamically in response to growth factor, hormonal, and environmental cues (328–330). Phosphorylation in response to serum- and glucocorticoid-regulated kinase 1 (SGK1) or AKT results in the cytoplasmic retention of FOXO proteins and, hence, reduced expression of FOXO target genes and increased proliferation (331, 332). Conversely, phosphorylation of cytoplasmic FOXO factors by other kinases, including Jun N-terminal kinase (JNK), promotes nuclear import (333).

In the human endometrium, FOXO1 is markedly up-regulated upon decidualization in vivo as well as in vitro (81). By contrast, FOXO3a expression is repressed upon endometrial differentiation, whereas FOXO4 appears not to be expressed in this tissue (334). FOXO1 is a downstream target of the BMP2-WNT4 signaling cascade in differentiating HESCs (203). However, FOXO1 knockdown in turn inhibits the expression of both WNT4 as well as BAMBI, a pseudoreceptor and potent inhibitor of BMP, activin, and TGFβ signaling pathways (335). The expression of FOXO1 is also regulated by Twist-related protein 1 (TWIST1), a basic helix-loop-helix transcription factor modestly induced in decidual cells (336–338). Yet knockdown of FOXO1 also inhibits the induction of TWIST1 and the related transcription factor TWIST2 in differentiating HESCs (102). Thus, FOXO1, in decidualizing HESCs, targets genes involved in the regulation of its own expression and activity (339).

FOXO1 is indispensable for the induction of differentiation markers, such as PRL, IGFBP-1, and LEFTY2, in decidual cells (102, 340). It also regulates apoptosis in
response to progestin withdrawal. FOXO1 resides predominantly in the nuclei of HESCs treated with a cAMP analog. Cotreatment with progestins promotes partial translocation and accumulation of this transcription factor in the cytoplasm of decidualizing HESCs. Conversely, withdrawal of the progestin results in rapid nuclear reaccumulation of FOXO1, enhanced expression of the pro-apoptotic Bcl-2-like protein 11, and increased apoptosis of decidualizing cells (334). Interestingly, silencing of FOXO1 expression in differentiating stromal cells completely abrogates apoptosis induced upon progestin withdrawal, indicating that decidualizing HESCs become dependent upon progesterone signaling for survival through partial cytoplasmic translocation and thus inactivation of FOXO1 (334, 341). In other words, a key function of uterine FOXO1 in humans and other higher primates may be to couple spontaneous decidualization to menstrual shedding of the endometrium in response to falling circulating progesterone levels.

d. HOX proteins. The HOX genes are developmental genes that cluster in 4 genomic loci. Within a given HOX cluster, genes are expressed sequentially and act on successive segments along the anterior-posterior axis in the developing embryo (342). Of the 13 paralogs in the HoxA cluster, Hoxa9, -10, -11, and -13 are expressed along the fetal paramesonephric duct in mice. After birth, the spatial pattern of Hox expression is maintained in the female reproductive tract with Hoxa9 being expressed in the Fallopian tubes, Hoxa10 in the uterus, Hoxa11 in the uterus and cervix, and Hoxa13 in the upper vagina. This pattern of expression is maintained in the human (343). Both Hoxa10- as well as Hoxa11-deficient female mice are sterile (344, 345), due to implantation failure and a deficient decidual reaction (346, 347). In human endometrium, HOXA10 and HOXA11 expression peaks during the midluteal phase, thus coinciding with the window of implantation (348, 349). In cultured HESCs, HOXA10 and HOXA11 expression is stimulated by estrogen or progesterone. The effects of these steroids appear to be additive and further enhanced by RLN (349–352). Knockdown of HOXA10 attenuates some (eg, PRL and IGFBP-1) but enhances other (eg, IL-11 and IL-15) decidual factors in culture (26).

HOX proteins function as transcription factors, binding DNA through a conserved sequence, the homeobox. Proteomic analysis has identified Fkbp4 as a downstream target of Hoxa10 in the peri-implantation mouse uterus (277) and, as already mentioned, this immunophilin is indispensable for implantation and decidualization in mice (353). In decidualizing HESCs, HOXA10 targets numerous cell cycle genes that are regulated in an opposite manner by FOXO1. This suggests that HOXA10 enables proliferation in the stromal compartment before and during the process of differentiation, a response counterbalanced by the antiproliferative actions of FOXO1 (100). On the MER20 enhancer of the decidual PRL promotor, HOXA11 acts as a transcriptional repressor but is converted to a strong activator in the presence of FOXO1 (354).

e. 3-Ketosteroid nuclear receptors. All members of the 3-ketosteroid receptor (NR3C) subfamily of nuclear receptors are expressed in HESCs, including GR (NR3C1), mineralocorticoid receptor (MR, NR3C2), PGR (NR3C3), and androgen receptor (AR, NR3C4). Not only do the circulating levels of the various hormonal ligands, ie, progesterone, cortisol, aldosterone, and androgens, respectively, rise in early pregnancy (355–357), induction of various steroidogenic enzymes increases local bioavailability of different ligands in decidualizing endometrium. For example, progesterone drives the expression of 11β-HSD1, which is reinforced upon subsequent conversion of inert cortisone to active cortisol. This positive feedback mechanism renders HSD11B1 one of the most highly induced genes upon HESC differentiation (29, 102) and, importantly, intrinsically links progesterone to cortisol signaling at the fetomaternal interface. Tissue androgen levels are also higher in secretory than proliferative endometrium, reflecting increased local conversion of androstenedione to testosterone (137, 358, 359).

The pattern of AR expression in the endometrial stromal compartment follows that of PGR, with the highest levels in proliferative endometrium and declining expression as the secretory phase unfolds (360). Functional analysis has shown that loss of AR protein in the stromal compartment during differentiation is primarily the consequence of the imposition of a translational block, mediated by progesterone-dependent induction of the poly(C)-binding protein 1 that targets AR transcripts (361). In agreement, studies in primates have shown that progesterins inhibit endometrial AR expression, whereas antiprogestins, such as RU486 or ZK 137316, induce AR expression in epithelial cells and enhance AR levels in the stroma (362). In cultured HESCs, AR protein is downregulated by cAMP and progesterone, although this is reversed upon cotreatment with androgens (103, 363). GR is expressed throughout the cycle, more so in the stroma than in the epithelial compartment, with levels rising dramatically during menstruation. MR is also present in both cellular compartments throughout the cycle (364, 365). In decidualizing HESCs, however, GR levels gradually decline and there is a reciprocal upregulation of MR as the differentiation process progresses (29).
Members of the NR3C subfamily display significant amino acid homology in their ligand- and DNA-binding domains (366). This structural homology accounts for the degree of promiscuity in terms of ligand binding between members. For example, cortisol activates GR as well as MR, progesterone is known to bind MR (367), and medroxyprogesterone acetate (MPA), a synthetic progesterin widely used to decidualize HESCs, is a potent activator of not only PGR but also of AR and GR (103, 368, 369). This degree of incongruous ligand binding activity combined with significant homology in the DNA binding domains suggest that NR3C members may transcriptionally control overlapping gene networks in decidualizing HESCs. Knockdown studies combined with genome-wide expression profiling demonstrated that this is not the case. In an initial study, the effect of PGR vs AR knockdown on decidual gene expression was compared. Interestingly, PGR knockdown deregulated approximately 9 times more genes than AR silencing (860 vs 92 genes, respectively). Only 19 genes were identified that were regulated in a similar manner by both PGR and AR (103). In a subsequent study, decidualizing HESCs were transfected with siRNA targeting either GR or MR and then subjected to microarray analysis. HESCs were decidualized for 4 days with a cAMP analog, progesterone, and cortisol. The rationale was that induction of endogenous 11β-HSD1 activity in differentiating HESCs would convert cortisol to cortisol, thus activating GR as well as MR. A total of 179 and 107 GR- and MR-dependent genes, respectively, were identified as well as 60 genes regulated by both nuclear receptors (29). Gene ontology annotation showed that a preponderance of AR-dependent genes is involved in cytoskeletal organization and cell cycle regulation (29, 103). This fits well with the observation that the antiandrogen hydroxyflutamide delays implantation in the rat and suppresses decidualization in pseudopregnant animals (370). MR target genes are strongly implicated in lipid droplet formation and retinoid metabolism, suggesting a role for this nuclear receptor in histotrophic support of the early conceptus (29).

By contrast, GR functions primarily as a transcriptional repressor in decidualizing cells and is important for limiting the expression of Krüppel-associated box zinc-finger transcription factors involved in heterochromatin formation (29, 371). Not unexpectedly, the impact of PGR silencing in decidualizing HESCs far exceeds that of other NR3C family members. A significant number of PGR-regulated genes in decidual cells code for membrane-bound receptors and intermediates in various signal transduction pathways. Furthermore, among the genes repressed by PGR are several that code for MMPs, death receptors of the TNF receptor superfamily, apoptosis mediators, oxidative stress defenses, and DNA repair enzymes. Thus, the critical dependency of the fetomaternal interface on sustained progesterone signaling is mediated at least in part by 1) PGR-mediated repression of factors involved in tissue destruction and 2) reprogramming of pathways activated by growth factors, cytokines, and other cues that control decidual gene networks (103).

f. Orphan nuclear receptors. Approximately half of the 48 members of the nuclear receptor superfamily has known natural ligands, which include steroid and thyroid hormones, lipids, and oxysterols, whereas the remainder is made up by orphan receptors (372). Several of these orphan receptors have been implicated in decidual transformation of HESCs. For example, liver receptor homolog-1 (LRH-1; encoded by the NR5A2 gene), is a key transcriptional regulator of genes involved in energy homeostasis, lipid metabolism, and steroidogenesis (373, 374). Intriguingly, experiments in mice that lack Nr5a2 in Pgr-expressing tissues revealed that this nuclear receptor is critical not only for postovulatory progesterone production in the corpus luteum but also for full decidualization of the uterus in pregnancy. Consequently, pregnancy in conditional Nr5a2-knockout mice, which is possible only with exogenous progesterone supplements, invariably leads to fetal demise (375). In human endometrium, LRH-1 is predominantly expressed during the proliferative phase, and levels decline after ovulation, yet depletion of LRH-1 in HESCs by siRNA perturbs the expression of various decidual markers (375).

The chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII, encoded by NR2F2) is a critical regulator of cell-fate specification, energy metabolism, angiogenesis, and reproduction (376). This nuclear receptor is expressed exclusively in stromal cells, yet it is essential to attenuate estrogen-dependent gene expression at implantation (377). Conditional uterine knockout of COUP-TFII in mice leads to infertility caused by implantation and decidualization defects (378). Intriguingly, the implantation defect can be reversed by a pure ESR1 antagonist (ICI 182,780), although subsequent placental formation remains defective (379). Stromal COUP-TFI expression in the human endometrium is most pronounced in the proliferative phase but decreases rapidly after ovulation, at least in the superficial endometrium (380). In primary cultures, several inflammatory genes are upregulated upon knockdown of COUP-TFII in differentiating HESCs, suggesting an important role for this orphan receptor in limiting the initial proinflammatory response upon decidualization (380). Interestingly, COUP-TFII is not only regulated by various morphogens (381), but silencing of this transcriptional regulator in decidualizing HESCs per-
turbs the expression of various WNT family members (380).

Two additional orphan nuclear receptors have been implicated in the decidual process. Estrogen-related receptor α (ERRα or NR3B1) is expressed in all endometrial cell compartments, and expression at the transcript level increases in the latter half of the secretory phase of the cycle. NR3B1 regulates genes involved in mitochondrial biogenesis, gluconeogenesis, oxidative phosphorylation, and fatty acid metabolism, and not surprisingly, its activity is enhanced in decidualizing HESCs, again underscoring the role of the decidua in energy production in early pregnancy (382). Pharmacological inhibition of NR3B1 in HESCs impairs induction and maintenance of the decidualized phenotype (382). Along similar lines, NUR77 (NR4A1), an important transcriptional regulator of glucose metabolism in liver and muscle, is upregulated in decidualizing HESCs, and knockdown perturbs the expression of decidual PRL (383). In the mouse uterus, Nur77 expressed in endothelial cells critically regulates endometrial vascular permeability and tissue edema before implantation (384).

**g. Signal transducers and activators of transcription.** A common signaling pathway used by many cytokines involves recruitment and activation of Janus kinases (JAKs) to the liganded cytokine receptor and subsequent phosphorylation and nuclear translocation of signal transducers and activators of transcription (STATs). Suppressor of cytokine signaling (SOCS) proteins are key negative regulators of the JAK-STAT pathway (385). It has been demonstrated that STAT3, 1 of 7 mammalian STAT isoforms, is the downstream regulator of IL-11 signaling in decidualizing HESCs. Levels of STAT3 as well as SOCS3 are elevated in decidualized cells, with the former being progestosterone- and the latter CAMP-dependent (386). Moreover, STAT3 has been identified as an essential mediator of HESC differentiation driven by C/EBPβ (323). Silencing of STAT3 or overexpression of SOCS3 in HESCs retards decidualization, emphasizing the critical role of STAT signaling in this process (323, 386). The level of activated STAT5 increases with decidualization and enhances transcription of the decidual PRL promoter (387). Conversely, IFNγ stimulates STAT1 expression, phosphorylation, and translocation to the nucleus, which in turn potently represses decidual PRL promoter activity. On the other hand, there is evidence that IFNγ may stimulate the expression of indoleamine 2,3-dioxygenase (IDO), the tryptophan-catabolizing enzyme essential for maternal immune tolerance to the fetal allograft (388).

**h. The tumor suppressors p53 and promyelocytic leukemia zinc finger (PLZF).** The tumor suppressor protein p53 is a transcription factor that plays a fundamental role in protecting the genome from genotoxic insults. Under physiological circumstances, the p53 mRNA is constitutively expressed, but the protein is nearly undetectable in most cell types because it is subject to rapid proteasomal degradation. However, in response to stress or DNA damage, the p53 protein is stabilized, rapidly accumulates in the nucleus, and initiates events leading to cell cycle arrest and DNA repair or to apoptosis, thus eliminating genotypically aberrant cells from the organism (389–391). In a large proportion of human tumors, p53 is mutated and functionally defective. This is accompanied by increased protein stability, thus rendering the mutated protein immunohistochemically detectable in cancer cells (392).

Secretory-phase HESCs have the unusual feature that p53 protein is readily detectable in their nuclei, although the accumulated protein is wild-type. In cultured cells, p53 is massively upregulated upon CAMP-induced decidualization, due to protein stabilization. The kinetics of induction are not those of an acute stress response but mirror the delayed increase of decidual markers after 2 to 4 days of decidualizing treatment. Upon withdrawal of the stimulus, the cells dedifferentiate morphologically and lose expression of PRL and IGFBP-1 along with disappearance of p53 protein (92). Homozygous deletion of p53 in female mice results in significantly reduced implantation, pregnancy rates, and litter sizes. This is partially mediated by reduced levels and function of LIF in the mutant uteri (393).

In vitro decidualization of HESCs in response to CAMP analog, estradiol, and MPA treatment was found to be associated with an initial cell cycle arrest in G0/G1, and arrest in G2/M phases at later stages, and involved members of the p53 pathway (394). One of the p53 target genes, growth arrest- and DNA damage-inducible protein of 45 kDa (GADD45α) (395), is also highly expressed in secretory-phase endometrium (396–398). GADD45 proteins are multifaceted factors implicated in the regulation of diverse stress responses and are presumed to serve as gatekeepers capable of killing cells that are unable to repair damaged DNA (399). A potential role of p53 and GADD45α in decidualization might be to halt proliferation and facilitate differentiation in the stromal compartment. Furthermore, p53 and GADD45α could also play a role in safeguarding genomic stability of decidual cells during placentation, when dramatic changes in oxygen tension induce a burst of harmful reactive oxygen species (400). Remarkably, p53 shares many functional features and target genes with FOXO1, rendering them partners to survey genomic integrity in the decidua (401).
Although p53 in decidualizing HESCs is exquisitely cAMP-responsive and regulated at the protein level, the transcription factor PLZF (also known as ZF145 or ZBTB16) is solely induced by progesterone or glucocorticoids at the transcriptional level (402). Yet it shows a similar pattern of endometrial expression, being massively increased in the nuclei of decidualized HESCs in vivo (402). PLZF has gained its name for being identified in acute promyelocytic leukemia where a reciprocal chromosomal translocation results in fusion proteins between PLZF and the retinoic acid receptor α (403). PLZF belongs to the large family of POZ domain and Krüppel zinc finger DNA-binding proteins and inhibits transcription by recruiting repressor proteins such as silencing mediator for retinoid and thyroid-hormone receptors (SMRT), nuclear receptor corepressor (N-CoR), Sin3, and histone deacetylase 1 (HDAC1) (404, 405). It exerts antiproliferative actions, eg, by suppressing the cyclin A promoter, and can confer resistance to apoptosis by inhibiting expression of BH3 interacting domain death agonist, a proapoptotic member of the Bcl-2 family (406, 407).

Unopposed activity of p53, as a result of cAMP signaling, would favor proapoptotic pathways in decidualizing HESCs. Progesterone-dependent upregulation of PLZF, in turn, promotes survival. FOXO1, as the third player, is positioned at the crossroads of apoptosis and survival, being upregulated by cAMP-dependent signals but inactivated to a large proportion by progesterone-dependent nuclear exclusion. Progesterone is thus tipping the balance in cell fate decisions in the endometrium at the cusp of menstruation or pregnancy (341).

i. Other transcriptional regulators. The composite transcription factor activating protein 1 (AP-1) serves as a major regulator of cell proliferation as it integrates various mitogenic signals. The AP-1 complex is composed of homo- or heterodimers of Jun, Fos, and activating transcription factor proteins, members of the bZIP family of transcription factors. The Jun family consists of c-Jun, JunD, and JunB, whereas the Fos family comprises c-Fos, FosB, Fra-1, and Fra-2 (408). In the endometrium, c-Fos and c-Jun expression is estrogen-dependent and confined to proliferative and early- to midsecretory endometrium (409). However, JunD and Fra-2 protein expression is markedly increased in secretory-phase endometrium and in the decidua of early pregnancy. Furthermore, JunD and Fra-2 have been shown to enhance decidual PRL promoter activity upon binding to AP-1 responsive elements in the upstream promoter region (410).

Ets1 belongs to the ETS transcription factor superfamily that is defined by a highly conserved 85-amino-acid DNA binding motif. ETS transcription factors serve as nuclear effectors of multiple signal transduction cascades and regulate a broad spectrum of cellular processes (411). Ets1 is highly induced in decidualizing HESCs in culture, and overexpression of Ets has been shown to stimulate decidual PRL gene expression through binding to an ETS motif located in the proximal promoter region (338).

There are 4 known members of the specificity protein (Sp) family of zinc finger transcription factors. Sp1, Sp3, and Sp4 proteins bind with similar affinities to a GC-rich DNA motif that is found in the promoter regions of many genes, including housekeeping genes. Sp1 and Sp3 are ubiquitously expressed, and there is evidence that, depending on the promoter context, Sp3 functions as a competitive repressor of Sp1-dependent transcription (412). In the human endometrium, Sp1 levels increase in perivascular stromal cells during the secretory phase of the menstrual cycle, whereas Sp3 expression decreases in HESCs upon decidualization (413). The expression of several decidual-specific genes, such as F3 (encoding TF), PAI1, and IGFBP1, has been shown to be regulated by the cellular Sp1 to Sp3 ratio (414).

Another factor gaining prominence in the decidual process is GATA binding protein 2. In mice, this transcription factor is expressed in luminal and glandular epithelium during the implantation window and induced upon decidual transformation of the stroma (415). Furthermore, uterine-specific Gata2 deletion in mice lowers PGR expression and is associated with defects in implantation and decidualization (416). Intriguingly, although GATA2 is not induced upon decidualization of HESCs, silencing of this transcription factor attenuates the induction of PRL and IGFBP1 without affecting PGR transcript levels (417).

4. Epigenetic regulation

There is growing evidence that the responsiveness to hormonal cues is dependent upon genome-wide remodeling of the chromatin structure of HESCs, which in turn enables PGR and other transcription factors to gain access to the decidua-specific regulatory circuitry. Accumulating evidence indicates that cycle-dependent transformation of the endometrium is indeed associated with profound changes in the expression of epigenetic modifiers and chromatin modifications (418, 419). In culture, transition from a proliferative to a decidual phenotype coincides with altered expression of 33 epigenetic effectors, including histone-modifying enzymes, histone-binding proteins, histone variants, CpG-binding proteins, and DNA methyltransferases (420). For example, the histone methyltransferase enhancer of Zeste homolog 2 (EZH2) is gradually lost upon decidualization of HESCs, which in turn results in declining levels of trimethylated lysine 27 of hi-
stone 3 (H3K27me3) at the proximal promoters of key decidual marker genes such as PRL and IGFBP1. Furthermore, loss of H3K27me3 at these sites is associated with a reciprocal enrichment in acetylation of the same lysine residue, indicating active remodeling from a repressive to a transcriptionally permissive chromatin. Genomewide mapping demonstrated that loss and gain of the repressive H3K27me3 mark in decidualizing cells occur at loci enriched for genes involved in cellular responsiveness to stimuli and growth/cell proliferation, respectively (105).

At a functional level, trichostatin A, a histone deacetylase inhibitor, enhances PRL and IGFBP1 expression in a dose-dependent fashion in differentiating HESCs (421). Furthermore, trichostatin A induces acetylation at the TIMP1 and TIMP3 promoters and increases their expression, an effect that also occurs with differentiation of HESCs in vitro (422). Another study reported that treatment of undifferentiated HESCs with the DNA methyltransferase attenuates TIMP1 and TIMP3 expression in a dose-dependent fashion in differentiating HESCs (421). The same investigators also focused on the downregulation of DNMT3B in decidualizing cells and demonstrated that exogenous expression of this de novo DNA methyltransferase attenuates IGFBP1 expression (424). Furthermore, inhibition of DNA methylation in the pre- and postimplantation phase in the mouse significantly disturbs decidualization and leads to loss of embryos (425). However, a recent genome-wide methylation analysis of HESCs revealed that decidualization is associated with hypomethylation at relatively few CpG sites (417).

Cooperative binding of transcriptional factors requires accessible chromatin, which is largely devoid of nucleosomes. Exhaustive genome-wide mapping of the accessible chromatin landscape as part of the ENCODE project has shown extraordinary diversity between different cell types and tissues (426). These cell-specific differences in chromatin structure determine the ability of sequence-specific transcription factors to bind cis-regulatory elements including promoters, enhancers, insulators, silencers, and locus control regions (427). For example, chromatin immunoprecipitation (ChIP) followed by massive parallel sequencing (ChIP-seq) identified 31,457 and 7034 PGR-binding sites in T47D breast cancer cells and primary leiomyoma cells, respectively (428). The complete set of cis-elements occupied by a transcription factor in the genome is referred to as the cistrome. Interestingly, a comparison of the PGR cistromes in breast cancer and leiomyoma cells identified only 1035 overlapping sites. Another study reported that most PGR binding regions are located more than 10 kb from the TSS of regulated genes.

In fact, less than 4% of regulated genes exhibit PGR binding within 1 kb of TSSs (429). Even in the absence of ligand, a significant proportion of PGRs is bound to DNA. For example, PGR was found to occupy 6367 sites in uteri of ovariectomized mice in the absence of progesterone stimulation. Acute exposure of mice to progesterone increased PGR binding to DNA 3-fold (415). Earlier studies also inferred a transcriptional role for the unliganded PGR in HESCs based on the observation that knockdown or treatment with a selective PGR antagonist is sufficient to inhibit cAMP-dependent decidualization in the absence of progesterone (103, 246).

Taken together, these studies indicate that the epigenome of HESCs is dynamically regulated as the decidual process unfolds. Yet, considering the sheer complexity of the epigenetic code, much more work is needed to comprehensively map the DNA and histone modifications associated with endometrial differentiation.

5. Post translational modifications and convergence of signaling pathways

Activation of the cAMP and progesterone signaling pathways has profound ramifications for signal transduction through other pathways. This is effected, on the one hand, through induction of autocrine and paracrine factors and, on the other, through altered expression of pathway intermediates and modulators. For example, treatment of primary HESC cultures with a cAMP analog and a progesterin inhibits the expression of several genes encoding for components upstream of JNK while simultaneously upregulating MAPK phosphatase 1. Consequently, stress-induced signaling through the JNK as well as p38 pathways is firmly disabled upon differentiation of HESC into decidual cells (104). Decidualization is also associated with inhibition of AKT (also known as protein kinase B) and reciprocal activation of SGK1 (332). This switch from AKT to SGK1 activity in differentiating cells is intriguing because both kinases are not only closely related but also controlled by identical upstream regulators: phosphatidylinositol 3-kinase, phosphoinositide-dependent kinase 1 (PDK1) and mammalian target of rapamycin complex 2 (mTORC2) (430). Other major pathways and kinases activated in differentiating HESCs include the WNT/β-catenin and JAK-STAT pathways (203, 323, 386, 387), ERK1/2 (103, 431), and c-Src (432, 433). Transfection of dominant-negative c-Src into HESCs, decidualized with estradiol plus progesterone, prevents morphological differentiation and induction of decidual marker genes (434). In the pregnant mouse uterus, prominent c-Src activation occurs and is obligatory for decidualization (435). Because this tyrosine kinase directly interacts with PGR...
(436), it is likely that progesterone signaling is deregulated in c-Src–deficient uteri.

These signaling pathways and kinase cascades ultimately converge on downstream transcription factors and their coregulators (Figure 3), thus rendering decidual gene expression responsive, or refractory, to specific signal inputs and environmental cues. Besides phosphorylation, the activity of transcription factors is further regulated by a host of other posttranslational modifications, including glycosylation, ubiquitination, nitrosylation, methylation, acetylation, and sumoylation. Protein posttranslational modification increases the functional diversity of the proteome by the covalent addition of functional groups or peptides. Consequently, differentiation-specific changes in the kinetics of these enzymatic reactions represent a mechanism to alter the behavior and activity of a host of targets in a coordinated manner. A case in point is the change in cellular sumoylation-desumoylation equilibrium upon decidualization of HESCs. Sumoylation, which denotes the covalent modification of proteins with small ubiquitin-related modifier (SUMO) proteins, is implicated in an array of cellular processes, including transcription regulation, DNA repair and stress responses (437). It is mediated by sequential activation of an E1-activating enzyme, the ubiquitin-conjugating enzyme E2I (also termed UBC9), and several E3 protein ligases that confer substrate specificity. Once a target is sumoylated, the modification can be rapidly reversed through the activities of sentrin-specific proteases (SENPs). Increased cAMP levels in differentiating HESCs trigger a gradual decline in global cellular sumoylation levels and redistribution of SUMO-1-modified proteins into distinct nuclear foci (438). Notably, this global hyposumoylation response is accounted for by altered expression of various E3-conjugating enzymes and SENPs, including simultaneous down-regulation of the E3 ligase protein inhibitor of activated STAT1 (PIAS1) and upregulation of SENP2. An important functional consequence of global hyposumoylation and altered expression of key enzymes is a marked decrease in ligand-dependent SUMO1 modification of key nuclear receptors, such as PGR and AR, which in turn greatly enhances the responsiveness of decidualizing cells to steroid hormones (103, 438). In fact, PIAS1 knockdown, which disables PGR-A sumoylation, renders HESCs partially responsive to progesterin treatment without a need to simultaneously activate the cAMP pathway (438).

IV. The Role of the Decidual Process in Embryo Implantation

A. The implantation window

A prevailing concept in reproductive biology is that the endometrium must transiently acquire a receptive phenotype to ensure that a competent blastocyst embeds in an optimal uterine environment (439–441). During this brief period, known as the window of implantation, the endometrium expresses an evolutionarily conserved repertoire of genes that enables a series of key events to take place, starting with 1) the positioning of the embryo near the fundus of the uterus; 2) absorption of uterine fluid, luminal closure, and apposition of the blastocyst on the endometrial surface epithelium; 3) stable adherence to the apical surface of luminal epithelial cells; 4) penetration through the luminal epithelium and its basal lamina, and finally, 5) invasion of the stroma (165, 442, 443).

For obvious reasons, the molecular details underpinning this process are derived from animal models. These studies have been exhaustively reviewed recently (444, 445). In mice, day 1 of pregnancy denotes the day of the vaginal plug. The endometrium then transits from a pre-receptivity (days 1–3) to a receptive (day 4) and finally to a refractory phase (day 5 onward) (446, 447). In humans, the receptive phenotype coincides with the midluteal phase (days 20–24) of a regular cycle, thus 6 to 10 days after the preovulatory LH surge (448, 449). This fits well with the observation that only between 5 and 7 days after the LH surge can free-floating embryos be efficiently retrieved from the uterine cavity by flushing (450). The earliest direct evidence of an implanting human embryo came from histological examination of a hysterectomy specimen obtained from a 38-year-old woman with a history of 10 previous pregnancies resulting in 9 full-term live births. The conceptus with an estimated postfertilization age of 7 days had eroded the endometrial epithelium but barely penetrated the endometrial stroma (451).

By measuring ovarian hormone metabolites and hCG on daily first-morning urine samples from fertile couples, Wilcox and colleagues (452) demonstrated that 84% of conceptions can be detected between 8 and 10 days after ovulation. Among the 102 pregnancies identified on day 9 after ovulation, 13% ended in an early pregnancy loss. This proportion rose to 26% with implantation on day 10, to 52% on day 11, and to 82% after day 11. Other studies have shown that a delayed rise in hCG levels is associated with smaller fetal size in the first trimester and later births in ongoing pregnancies (453, 454). Furthermore, a recent population-based prospective cohort study reported that impaired first-trimester fetal growth increases the likelihood of an adverse cardiovascular risk profile in school-age children (455). Taken together, these clinical studies strongly suggest that the timing of implantation has a major impact on the likelihood of miscarriage and, in ongoing pregnancies, on the future health of the offspring.

Failure of the endometrium to achieve a receptive state is thought to be a major cause of infertility as well as the
rate-limiting step in assisted reproductive technology. Consequently, exhaustive efforts have been made to identify biomarkers of endometrial receptivity using a variety of approaches, including histological dating (456), ultrastructural analysis (457), high-throughput immunohistochemistry (458), proteomics of endometrial biopsies (459, 460), and cytokine, proteomic, and lipidomic analyses of endometrial secretions obtained by uterine lavage (461–466). In addition, microarray technologies have been widely employed to assess prereceptive vs receptive endometrium (397, 467–472). The results of these gene expression profiles are highly disparate, and only 2 genes were common to all 6 studies, SPPI (coding for osteopontin) and IL15 (473). Furthermore, aside from a handful of putative biomarkers, the reported transcriptional signatures of the receptive endometrium are seemingly unconnected to either the endometrial proteomic or secretome profiles (473).

Several explanations have been put forward to account for the failure so far to identify and validate clinically useful biomarkers predictive of a receptive endometrium. Commonly cited reasons include the relatively small sample size in many studies, differences in design and analytical platforms, and the inherent variability of endometrial gene expression not only between individuals but also from cycle to cycle in a given individual (474). Although these explanations have merit, there are probably more cogent reasons for why a window-of-implantation test has been elusive. For example, the idea that the postovulatory endometrium functions as a binary switch (receptive/non-receptive) originated from observations in polytocous species, primarily mice. Here, reproductive success is based on rapid breeding cycles, synchronized implantation of multiple embryos, large litter sizes, and selection of pups after birth. With an average lifespan of 18 months, an ability to breed as often as every 20 days, and an average of 10 pups per litter, a single female mouse can produce more than 300 offspring in her lifetime. This astounding level of implantation efficacy is achieved because a single endocrine signal not only functionally switches a progesterone-primed, prereceptive endometrium to a receptive state but also activates the implantation-incompetent or dormant preimplantation embryo. This obligatory maternal implantation signal consists of a transient rise in postovulatory estradiol production, leading to increased uterine catechol estrogen synthesis, which in turns renders the blastocyst competent for implantation (444, 447, 475, 476).

Unlike the situation in mice and other rodents, there is no evidence that a distinct endocrine cue controls receptivity in the human endometrium (Table 3). There is also no compelling evidence that human embryos are either capable of delaying implantation by entering a metabolically dormant state or that maternal estrogen metabolites render human blastocysts implantation-competent. Although estradiol levels also increase transiently during the midluteal phase of the cycle in humans and subhuman primates, there is no evidence that this rise is needed for implantation (477). The apparent loss, or at least dilution, of these critical implantation mechanisms is entirely in keeping with the fact that humans face very different reproductive challenges than mice. Reproductive fitness in humans primarily depends on the ability to accommodate, or reject, a highly invasive and often chromosomally abnormal conceptus (12, 478–480). In fact, a bewildering array of chromosomal errors has been detected in human embryos throughout all stages of preimplantation development, and the incidence is estimated to be an order of magnitude higher than in other nonprimate species. In excess of 70% of high-quality cleavage-stage IVF embryos have been shown to harbor cells with complex large-scale structural chromosomal imbalances, which are caused mostly by mitotic nondisjunction (481–483). As outlined below, accumulating evidence suggests that cyclic decidualization bestows essential functions on the endometrium that couple receptivity to active selection of embryos at implantation.

### B. Embryo invasion and encapsulation

The process of implantation involves extensive tissue remodeling and resembles in several aspects tumor invasion. Consequently, decidualization is intuitively viewed as a process that limits excessive trophoblast invasion, thus preventing pathologies such as placenta accreta. Yet, to form a functional placenta and to ensure the growth of the offspring, the decidua must equally promote and accommodate invasive trophoblast. These seemingly oppos-

<table>
<thead>
<tr>
<th>Embryonic characteristics</th>
<th>Mouse</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dormancy/diapause</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Chromosomal instability</td>
<td>Rare</td>
<td>Very prevalent</td>
</tr>
<tr>
<td>No. of implantation sites</td>
<td>±10</td>
<td>1–2</td>
</tr>
<tr>
<td>Invasiveness</td>
<td>Low</td>
<td>High</td>
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<td>Hemochorial placenta</td>
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<td>Deep</td>
</tr>
<tr>
<td>Maternal decidualization</td>
<td>Trigger</td>
<td>Implantation</td>
</tr>
<tr>
<td>Progesterone dependency</td>
<td>Yes</td>
<td>Endocrine cues</td>
</tr>
<tr>
<td>cAMP dependency</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cell polyploidization</td>
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</tr>
<tr>
<td>Terminal differentiation</td>
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</tr>
<tr>
<td>Menstruation</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cyclic regeneration</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
showed no signs of degeneration or apoptosis, indicating phoblast and decidual cells. Notably, decidual cells somal cell-cell contacts were observed between cytotro-

estravillous trophoblast was visible. Extensive desmo-

plants with chorionic villi (496). Within 96 hours, invaded interface has been assessed in cocultures of decidual ex-

cells (495). The ultrastructure of the early fetomaternal of embryo-derived PDGF-AA coincides with increased ex-

tome analysis of trophectoderm from human blastocysts implantation-competent blastocysts (494), and transcrip-
tion sequencing revealed a dramatic upregulation of the implanted embryo (498). Furthermore, next-gen-
eration sequencing revealed a dramatic upregulation of essential implantation and metabolic genes in murine uteri transiently exposed to spent culture medium of develop-
mentally competent human embryos. By contrast, this re-
sponse was entirely absent after exposure to culture me-
dium of low-quality human embryos. Instead, numerous other genes were perturbed, indicative of an endometrial stress response (480).

Several in vitro models have highlighted the particular aptness of decidualizing cells in sensing poor-quality hu-

man embryos. Teklenburg and colleagues employed a hu-

cman coculture model, consisting of single hatched blasto-
cysts cultured on 50 000 decidualizing HESCs, to identify key factors involved in the embryo-maternal cross-talk (12). The coculture supernatants were collected after 3 days and assayed for 14 implantation cytokines, growth factors, and chemokines. Expression levels were then compared with the levels produced by decidualizing cells in the absence of an embryo. Rather unexpectedly, the presence of a normally developing human blastocyst had no mea-
surable effects on the secretion of implantation factors by decidualizing cells. However, a marked response was noted when the embryo seemed to be arresting during the coculture period. The maternal response was character-
ized by selective inhibition of several ILs, CXCL11, and HB-EGF secretion. Repeat coculture experiments with undi-

fentiated HESCs yielded no response irrespective of embryo quality (12). In a follow-up study, these investi-
gators combined a modified wound-healing assay with time-lapse microscopy and found that decidualizing HESCs selectively migrate toward high-grade but not low-grade human embryos (494). The failure of decidualizing cells to home in on poor-quality embryos fits with the inhibition of HB-EGF and IL-1β, which are prominent regulators of HESC motility and embryo-maternal cross-talk (488).

The mechanism that imparts responsiveness of decidualizing HESCs to poor-quality human embryos is not understood. Microarray analysis identified HSPA8 as the most dysregulated decidual gene among 447 genes responsive to signals from developmentally impaired human embryos (480). HSPA8 (also known as HSC70, HSC71, HSP71, or HSP73) is a multifaceted constitutively expressed molecular chaperone that can represent up to 1% of total cellular protein content. It is involved in clathrin-mediated endocytosis, assembly of multiprotein complexes, transport of nascent polypeptides, and regulation of protein folding. It is also a major regulator of autophagy, especially chaperone-mediated autophagy (500). HSPA8 expression increases upon decidualization of HESCs, in parallel with the expanding endoplasmic reticulum and acquisition of a secretory phenotype (480). Hence, the importance of this molecular chaperone increases as differentiating HESCs expand their secretory machinery, which may explain why decidual but not undifferentiated cells are exquisitely adapted to sense compromised human embryos.

Although the molecular details are still sketchy, there is evidence to suggest that both human and murine embryos signal implantation competence by secreting serine proteases that cleave and activate the epithelial Na⁺ channel present on the apical border of luminal endometrial epithelial cells (EECs) (480). This in turn leads to an inward current, membrane depolarization, and Ca²⁺ entry into EECs through voltage-gated L-type Ca²⁺ channels. The resultant Ca²⁺ transients then phosphorylate and activate the transcription factor CREB, which in turn leads to upregulation of COX-2 activity and PGE2 release (501) (Figure 2). Importantly, the Ca²⁺ transients induced in EECs by developmentally competent human embryos were very short-lived, lasting approximately 5 minutes. In contrast, low-quality human embryos triggered prolonged and disorganized Ca²⁺ oscillations in EECs, which is speculated to reflect excessive or unopposed secretion of tryptic embryonic proteases (480).

Taken together, these observations suggest an important role for the luminal epithelium in transducing an embryo-derived implantation initiation signal, whereas cyclic decidualization of the underlying stroma may primarily serve to limit maternal investment in invasive but developmentally compromised human embryos.

D. Immunomodulation and maternal tolerance

The Nobel laureate Sir Peter Brian Medawar (502) is credited widely for articulating the paradoxical immunological relationship that exists between mother and fetus in pregnancy. Medawar was also the first to propose that survival of the allogeneic conceptus requires an evasive mechanism based on the concept of self/nonself recognition in classical transplantation biology. His views inspired the field of reproductive immunology, and several excellent reviews chart recent discoveries and progress (503–506).

Decidualizing stromal cells are often viewed as bystanders when it comes to the immunology of the maternal-fetal interface. This perception has been challenged profoundly by recent studies demonstrating that differentiating resident stromal cells act both as key gatekeepers and chief modulators of local immune cells, thus ensuring that the maternal-fetal interface is abundantly populated by uNK cells and specialist macrophages but not NK T cells, B lymphocytes, or uDCs (36, 507, 508). Tissue DCs are crucial initiators of immunogenic T cell responses to foreign antigens, a process that depends on migration of these activated antigen-presenting cells to the draining lymph nodes. Elegant studies in mice have shown that uDCs present in the decidua are unable to migrate from the tissue to the uterine lymph nodes (509, 510). Entrapment of uDCs, which minimizes the potential of immune surveillance of the conceptus, is thought to reflect the loss of lymphatic vessels, particularly around the spiral arteries, upon decidualization of the stroma (511). However, the loss of lymphatic vessels in the decidua is contentious (512, 513), and other mechanisms, such as ECM changes or the lack of chemokine gradients necessary for homing to lymphatic vessels, have been invoked to explain entrapment of uDCs in the decidua of early pregnancy (504, 509).

The gatekeeper function of decidual cells does not only minimize the risk of priming maternal T cells to paternal alloantigens expressed on trophoblast but also actively prevents influx of antigen-specific cytotoxic T lymphocytes, at least in the mouse decidua. This is mediated by silencing of decidual genes encoding key T helper type 1 cell- and cytokotoxic T lymphocyte-attracting chemokines, such as Cxcl9, Cxcl10, Cxcl11, and Ccl5 (504, 514). These genes are epigenetically silenced through accumulation of a repressive histone mark, H3K27me3, in their promoter regions, thus preventing reactivation upon treatment of cells with TNFα and IFNγ (514). Whether or not this mechanism also operates in the human uterus is as...
yet unknown, although it is striking that decidualization of human stromal cells involves a transient proinflammatory response that is followed by sustained downregulation of numerous chemokines and other inflammatory modulators (27). In addition, analysis of different stromal subpopulations isolated and cultured from midluteal human biopsies revealed that mature stromal cells secrete significantly lower levels of CXCL10 and CXCL5 when compared with matched perivascular MSCs, irrespective of whether the cells are decidualized or not (Murakami, K., and J. J. Brosens, unpublished observations).

Additional mechanisms have been implicated in T cell tolerance toward the allogeneic conceptus. For example, decidualizing stromal cells highly express galectin-1 (515–517), a multifaceted glycan-binding lectin strongly implicated in tumor immune evasion and autoimmune diseases (517–519). Galectin-1 is known to inhibit T cell proliferation and survival and attenuates expression of proinflammatory cytokines by activated T cells. Galectin-1–deficient mice exhibit higher rates of fetal loss compared with wild-type mice in allogeneic but not syngeneic matings. In this model of fetal antigen-mediated pregnancy loss, treatment with recombinant galectin-1 prevents miscarriage and restores maternal tolerance, at least partly through induction of tolerogenic dendritic cells and regulatory T cells (520). In addition, uNK cell–derived galectin-1 has also been shown to promote apoptosis of activated T cells in the decidua (521). There is evidence that human decidualizing stromal cells also express the tryptophan-catabolizing enzyme IDO (388). Tryptophan is a rare but essential amino acid required for cell proliferation. Studies in mice have revealed an essential role for IDO in pregnancy. Treatment of pregnant mice with an IDO inhibitor, 1-methyl-tryptophan, induces extensive inflammation, massive complement deposition, and hemorrhagic necrosis at the fetomaternal interface, resulting in the resorption of semiallogeneic fetuses (522, 523). This inflammatory response is not observed in syngeneic pregnancies, suggesting that IDO activity protects the fetus by suppressing T cell–dependent inflammatory responses, although whether this is accounted for by intrauterine or systemic effects is as yet unclear (504). Finally, decidualizing cells highly express Fas ligand, which induces apoptosis of activated T cells. Remarkably, decidualizing cells also express Fas (also termed CD95 or apoptosis antigen 1), the cognate cell surface receptor for Fas ligand, but auto-activation of the death-receptor signaling pathway is prevented by simultaneous induction of cellular FLICE-like inhibitory protein, a potent cytoplasmic inhibitor of the Fas pathway (398).

Decidual stromal cells are also pivotal in instructing local macrophages and NK cells, meaning that decidual cues bestow specialist functions on these immune cells. For example, uNK cells as opposed to peripheral NK cells are characterized by their reduced cytotoxicity and enhanced capacity to secrete a wide variety of chemokines, cytokines, and angiogenic molecules (524–527). Conditioned medium from decidual stromal cells supplemented with IL-15 and stem cell factor was shown to be sufficient to convert peripheral blood NK cells into a phenotype that resembles decidual NK cells (528). Furthermore, coculture with decidual stromal cells is sufficient to convert CD34+ hematopoietic precursors into phenotypic uNK cells (529). A recent study reported that a combination of hypoxia, TGF-β1, and a DNA demethylating agent attenuates the cytotoxicity of peripheral NK cells, increases the expression of VEGF, and bestows an ability on these cells to promote invasion of human trophoblast cell lines (530). These observations illustrate the plasticity of NK cells to adapt to a tissue-specific environment. Whether or not uNK cells actually originate from local precursors in the endometrium or are recruited from the periphery remains unclear, but their role in vascular remodeling, trophoblast invasion, and ultimately, fetal growth and well-being is unequivocally established (524, 531–535). In the first trimester of pregnancy, decidual macrophages and NK cells are found in close proximity of invading human extravilous trophoblasts (536). At this stage, macrophages make up approximately 20% to 30% of all decidual leukocytes (537, 538), express markers of alternative activation (539, 540), and in contrast to NK cells, remain present throughout pregnancy (541). Macrophages have been dubbed the most plastic cells of the hematopoietic system because of their functional diversity (542). This also applies to decidual macrophages, which consist of at least 2 distinct subpopulations as determined by the differential expression of the complement receptor CD11c (543). This cellular heterogeneity may account for the pleiotropic roles of decidual macrophages in effecting rapid tissue remodeling during pregnancy as well as conferring tolerance to invading semiallogeneic trophoblasts (544). Experimental evidence suggests that decidual macrophages inhibit NK cell cytotoxicity as well as cooperate with NK cells to induce immnosuppressing regulatory T cells (545, 546). Intriguingly, recent studies have shown that decidual stromal cells share these immunomodulatory functions and are equally capable of suppressing NK cell cytotoxicity and inducing regulatory T cells (547, 548).

In summary, decidualization of the endometrial stroma is increasingly viewed as the key process that accounts for the immunological paradox of pregnancy. Although some reproductive immunologists may well be skeptical of this claim, transplant biologist are embracing the emerging therapeutic opportunities and are exploring the use of de-
cidual stromal cells obtained from fetal membranes (i.e., HuF cells) for the treatment of severe steroid-refractory acute graft-vs-host disease (549).

E. Embryo rejection and menstruation

Another paradox is that decidualizing stromal cells are programmed both to resist a variety of environmental stressors in early pregnancy and to trigger tissue destruction in the absence of implantation or in response to a compromised conceptus (104, 550). It has been estimated that approximately 60% of all human embryos are disposed of in menstruation-like events before any telltale signs of pregnancy (551). Perhaps counterintuitively, bleeding associated with a pregnancy failure before 6 weeks gestation tends to be slightly longer but is less heavy than a woman’s typical menses (552).

Withdrawal of progesterone action from a decidualizing endometrium is the universal signal to induce menstruation or a menstruation-like event in case of early pregnancy loss. In this respect, duplication of an ancestral gene for the LH β-subunit in primate evolution was a critical event that enabled human embryos to produce hCG, which in turn rescues the maternal corpus luteum temporarily while awaiting the onset of placental progesterone production around 6 to 8 weeks of gestation (553, 554). Thus, decidual cells are a priori programmed to select against embryos that are perceived to lack fitness because of insufficient hCG production. However, additional mechanisms are likely at play to minimize maternal investment in an invasive but developmentally compromised human embryo. For example, by shutting down the expression of key implantation factors, decidual cells may induce a microenvironment that, directly or indirectly, interferes with trophoblast hCG production (555). Conversely, it is conceivable that proteotoxic signals emanating from developmentally impaired embryos interfere with PGR activity in decidual cells, thus triggering functional progesterone withdrawal and tissue breakdown regardless of the level of hCG production or circulating progesterone.

A detailed description of the tissue changes associated with menstruation and underlying paracrinology is beyond the remit of this paper but discussed in several excellent reviews (556–558). Suffice it to state that much of our understanding of the sequence of events that lead to menstrual shedding stems from the seminal study of Markee (559), in which endometrial tissue was transplanted autologously into the anterior eye chamber of rhesus monkeys. These experiments demonstrated that progesterone withdrawal triggers alternating episodes of vasodilation and vasoconstriction before bleeding from the transplants. Subsequently, these vasospasms have been attributed to increased prostaglandin and endothelin production and inferred to cause ischemic hypoxia in endometrial tissues (560), a conjecture vehemently contested in a recent study (561). Markee also observed intense shrinkage of the explant before bleeding, reflecting coordinated activation of proinflammatory cytokines, chemokines, and MMPs that precedes menstrual shedding (556–558). Another important observation that emerged from nonhuman primate studies, in this case the macaque, is that tissue breakdown and menstruation can be avoided if progesterone is added back within 36 hours of withdrawal (562, 563). Beyond this time point, add-back is without effect in this species. This suggests that menstruation is the end result of a step-wise process, characterized first by a gradual and reversible rise in inflammatory mediators, but once a threshold is reached, tissue destruction is inevitable.

A recent study provided compelling evidence that decidualizing stromal cells are responsible for the initial rise in inflammatory mediators in response to progesterone withdrawal (564), which becomes amplified and ultimately irreversible upon recruitment and activation of various leukocyte populations (neutrophils, eosinophils, basophils, mast cells, and macrophages). Withdrawal of progesterone from decidualizing stromal cells decreases levels of inhibitor of nuclear factor-κB (565). As already stated, FOXO1 is partly sequestered in the cytoplasm of decidualizing stromal cells. Withdrawal of progesterone induces rapid nuclear translocation of cytoplasmic FOXO1, activates BIM expression, a proapoptotic target gene, and induces cell death. Strikingly, FOXO1 knockdown is sufficient to completely abolish cell death induced by progesterone withdrawal (334). Induction of apoptosis in decidual stromal cultures upon progesterone withdrawal is, however, relatively modest (∼3-fold), although this may well suffice to destabilize the terminal spiral arteries in vivo and initiate bleeding.

Taken together, these studies indicate that decidualization in a nonconception cycle is a triphasic process (Figure 4), characterized initially by an acute proinflammatory response, which is then followed by a profound anti-inflammatory response, and finally reactivation of the inflammatory phenotype triggered by the fall in circulating progesterone before menstruation. It is tempting to speculate that in a failing conception cycle this triphasic decidual response sequentially controls endometrial receptivity, embryo biosensoring, and ultimate disposal of an unwanted conceptus in a menstruation-like event. In case
of a successful pregnancy, circulating progesterone levels do not fall in humans until after parturition. However, this does not exclude the possibility that the second proinflammatory response is initiated upon senescence of decidual cells, heralding activation of the parturition machinery. Credence for this conjecture comes from mouse studies demonstrating that uterine deletion of tumor suppressor Trp53 causes preterm birth due to premature decidual senescence (566).

F. Cyclic endometrial repair

Colin Finn (567, 568) was the first to argue that menstruation is nothing more than a nonadaptive consequence of uterine evolution. However, this does not exclude the possibility that the second proinflammatory response is initiated upon senescence of decidual cells, heralding activation of the parturition machinery. Credence for this conjecture comes from mouse studies demonstrating that uterine deletion of tumor suppressor Trp53 causes preterm birth due to premature decidual senescence (566).
ensuring that tissue repair, and presumably stem cell mobilization, always precedes pregnancy (576).

The actual regenerative process in the endometrium is far from understood and profoundly understudied. A rapid and crucial step is re-epithelialization, and areas of active shedding and repair are found in close proximity in menstrual endometrium (577). Based on scanning electron microscopy, glandular stumps protruding from the denuded surface are thought to be one source of luminal epithelial cells during menstrual regeneration (578, 579). However, compelling evidence from mouse models, examining either induced menstruation or postpartum repair, demonstrates that stromal cells undergoing mesenchymal-epithelial transition contribute to rapid regeneration of the epithelium (126, 127, 579, 580). This process is characterized by the appearance at the stromal-myometrial border of transitional cells that co-express epithelial (pan-cytokeratin) and stromal (vimentin) cell markers, which then migrate to the regenerative zone near the lumen as the repair process unfolds (127).

V. Clinical Reflections

A. Reproductive failure: an evolving paradigm

For much of the 20th century, the endometrium was viewed as an effector organ solely under the control of the hypothalamic-pituitary-gonadal axis. As a consequence, investigations into reproductive failure not attributable to overt uterine pathology were focused on clinical or subclinical endocrinopathies, such as polycystic ovary syndrome or luteal-phase defect. Along with the discovery of new regulatory pathways that control steroid actions, an expectation grew that specific molecular defects in the endometrium would account for implantation failure or recurrent pregnancy loss. Confidence in this paradigm was bolstered by genetic studies in mice, which provided incontrovertible evidence that implantation and decidualization are dependent on a number of key signaling molecules, receptors, transcription factors, and coregulators (Table 2). Yet, despite the wealth of knowledge gleaned from these model systems, as well as from the screening of clinical samples, it has proven frustratingly difficult to develop tests predictive of reproductive outcome or treatments that prevent pregnancy failure. A case in point is endometrial LIF, which is indispensable for implantation and decidualization in mice and possibly deregulated in infertile women, yet treatment with recombinant LIF was shown to decrease clinical pregnancy rate after IVF treatment in a randomized, placebo-controlled trial (181, 185, 581). The first study to demonstrate the existence of putative endometrial epithelial and stromal stem cells was published only a decade ago (110). Although seemingly innocuous and even unsurprising, this report led to the gradual realization that steroid hormone responses in the endometrium are highly dynamic and dependent on continuous cellular renewal and programming. Because of its regenerative capacity, the human uterus is intrinsically adaptable and capable of shifting its function to ensure reproductive success. Two prevalent disorders, endometriosis and recurrent miscarriage, aptly illustrate the contribution of aberrant cellular programming to impaired decidualization and, ultimately, reproductive failure.

B. Aberrant decidualization and endometriosis

Endometriosis, which affects 6% to 10% of women of reproductive age, is a leading cause of pelvic pain and subfertility. There is an association between the incidence of endometriosis and reproductive characteristics that increase pelvic exposure to menstrual effluent, such as early menarche, heavy periods, or short cycles (582, 583). These epidemiological observations lend support to Sampson's theory of retrograde menstruation as the cardinal cause of ectopic endometrial implants (584). However, retrograde menstruation is virtually ubiquitous, and it is not understood why some women develop endometriosis, whereas many do not. One plausible explanation is aberrant decidualization as this process controls endometrial shedding and, by default, the cellular and acellular constituents of menstrual fluid.

In fact, there is overwhelming evidence that decidualization of stromal cells is grossly impaired in endometriosis patients, both in the eutopic endometrium as well as in ectopic lesions (101, 282, 585–589). Arguably, the profoundly different microenvironment at ectopic sites will invariably have an impact on the cellular identity of transplanted stromal cells and, hence, their responsive-ness to deciduogenic signals. This is indeed the case as elegantly illustrated by genome-wide gene expression profiling and DNA methylation analysis of primary stromal cells isolated from either ovarian endometriomas or eutopic endometrium from endometriosis-free women (417). In an undifferentiated state, 4248 CpG dinucleotides, respectively, were differentially expressed and methylated between eutopic and ectopic HESCs. This wholesale reprogramming of the epigenome leads to simultaneous repression of key transcription factors that define the identity of eutopic HESCs (eg, ESR1, PGR, GATA2, and various HOX genes) and induction of other transcriptional regulators (eg, ESR2, SF1, and GATA6). Ergo, the response to deciduogenic signals is greatly blunted and deregulated...
in HESCs when compared with eutopic cells from endometriosis-free women (417).

The eutopic endometrium of patients with endometriosis is characterized by numerous cellular and biochemical alterations, as summarized in recent reviews (590–593), but the underlying drivers are not well understood. Intriguingly, animal models have shown that transplantation of eutopic endometrium to an ectopic site is sufficient to induce endometriotic lesions and, conversely, that induction of pelvic endometriosis disrupts the eutopic endometrium in a manner akin to the human situation (594–596). Endometrial differences between women with and without endometriosis are recapitulated and even accentuated in primary cell cultures, again inferring a pathological programming event. A striking characteristic is a severely blunted progesterone response in differentiating eutopic stromal cells. For example, Aghajanova and colleagues (282) reported that progesterone treatment of purified HESCs from disease-free patients alters the expression of 8, 62, and 172 genes after 6 hours and 2 and 14 days of stimulation, respectively (282). Uterine stromal cells from patients with mild endometriosis, however, responded to the same treatment regime by inducing the expression of only 0, 3, and 4 genes, respectively. These and other observations gave rise to the term progesterone resistance (597), which is somewhat misleading because endometriosis is also associated with altered gene expression in undifferentiated primary cultures as well as refractory responses to other differentiation signals, including cAMP and hCG (460, 588, 598, 599). Although as yet untested, it seems likely that the triphasic sequence of a normal decidual response is profoundly compromised in endometriosis, which in turn may account for the clinical association of endometriosis with implantation failure and menstrual disturbances, such as premenstrual spotting (600). Furthermore, the same pathological pathway may contribute to the increased risk of preterm labor in women with endometriosis (601–603).

Induction of surgical endometriosis in mice reduces the engraftment of bone marrow stem cells in the uterus, a process that is reversed upon regression of the lesions in response to treatment with an estrogen receptor modulator (604). This observation is intriguing because it suggests that the aberrant differentiation responses in the endometrium of affected patients are triggered by as yet ill-described deficiencies in the uterine stem cell niche. A provocative new hypothesis proposes that endometriosis originates from retrograde uterine bleeding soon after birth (605). Approximately 5% of newborns experience overt vaginal bleeding presumably triggered by withdrawal of maternal pregnancy hormones (606). This incidence mirrors the findings of an autopsy study, which reported that 5% of uteri of newborns show histological evidence of decidualization or menstruation (607). Taken together, it is conceivable that endometrial progenitor cells seeded in the pelvic cavity after overt neonatal menstruation remain largely dormant until menarche. Once reactivated, these very early lesions could, over cumulative cycles, diverge sufficient extrauterine stem cells away from the eutopic endometrium to affect decidual responses and menstrual events, thus establishing a feed-forward mechanism that reinforces the disease.

C. Aberrant decidualization and early pregnancy loss

Miscarriage, defined as the spontaneous loss of pregnancy before the fetus reaches viability, is the most common complication of pregnancy. One in 7 recognized pregnancies end in miscarriage during the first trimester and 1% to 2% fail between 13 and 24 weeks gestation. The incidence of early pregnancy loss rises to 15.2% in teenagers 14 years old or under, and 18.4% in women aged 40 years or older (608). Besides physical trauma and psychological morbidity (609), miscarriage increases the risk of adverse outcome in a subsequent pregnancy, including preterm delivery, premature preterm rupture of membranes, and low birth weight (610, 611). Furthermore, a recent meta-analysis concluded that miscarriage is associated with a greater risk of subsequent coronary heart disease (612).

Management of recurrent pregnancy loss (RPL) is particularly challenging. The American Society for Reproductive Medicine defines RPL as 2 or more consecutive pregnancy losses, whereas the European Society for Human Reproduction and Embryology has adopted a definition of 3 or more pregnancy failures (613, 614). Affected couples are routinely screened for various anatomical, endocrine, immunological, thrombophilic, and genetic risk factors, although the value of these investigations is highly contentious. In a majority of patients, no underlying associations are found, and conversely, many subclinical disorders or risk factors perceived to cause miscarriages are also prevalent in women with uncomplicated pregnancies (615). Embryonic chromosomal imbalances are estimated to account for approximately 50% of all miscarriages, but with each additional miscarriage, the incidence of euploidic fetal loss increases, whereas the likelihood of a successful pregnancy decreases. RPL patients are often treated empirically, commonly with a variety of immunomodulatory drugs based on the pervasive but unproven assumption that miscarriage is triggered by maternal immune rejection of an allogeneic fetus. Perhaps with the exception of progesterone (616), none of the
treatments for RPL has been shown to be unequivocally effective in well-designed and adequately powered clinical trials.

The emerging concept of active selection of human embryos at implantation provides a new ontological dimension to early pregnancy loss. To sense, support, or reject implantation, decidualizing cells surrounding the conceptus must expand their endoplasmic reticulum and be fully secretory (555), a phenotype that follows on from the resolution of the initial proinflammatory differentiation phase. As is the case for endometriosis, HESCs obtained from RPL patients also mount an aberrant decidual response when differentiated in culture. However, in contrast to the highly blunted response observed in endometriosis, recurrent miscarriage is associated with a prolonged and highly disordered proinflammatory response (27). This was aptly illustrated in a recent study that measured the secretion of IL-33 and its soluble decoy receptor sST2 in primary HESC cultures from RPL and non-RPL subjects. IL-33 is a key immune regulator and potent proinflammatory danger signal (alarmin) released in response to trauma or infection (617). However, IL-33 is also secreted upon decidualization of HESCs, triggering autocrine and/or paracrine activation of its cell surface receptor ST2L, which in turn drives an acute-phase response characterized by coordinated induction of ILs, chemokines, C-reactive protein, and other inflammatory mediators. Downregulation of ST2L and simultaneous induction of sST2 ensures that this initial proinflammatory decidual response is intrinsically self-limiting. Congruently, analysis of 16 non-RPL cultures showed that IL-33 secretion is 10-fold higher on day 4 compared with day 10 of decidualization, but strikingly, a reversed pattern was observed in 15 RPL cultures. Furthermore, the induction of the anti-inflammatory decoy receptor sST2 upon 10 days of differentiation was significantly blunted in RPL cultures compared with control cultures. To examine the functional consequences on the implantation process, mouse uteri were transiently flushed with conditioned medium of undifferentiated and decidualizing HESC cultures before embryo transfer. Strikingly, secreted factors from decidualizing RPL cultures not only prolonged the window of receptivity but also increased the incidence of pathological implantation sites, characterized by focal bleeding, immune cell infiltration, and fetal demise (27). In addition to a prolonged and disordered proinflammatory response, differentiating HESCs from RPL patients are characterized by lower induction of decidual marker genes, increased vulnerability to oxidative apoptosis, aberrant responses to hCG, and failure to discriminate between high- and low-quality human embryos in cell migration assays (11, 494, 618).

These observations predict that a pronounced or excessive decidual response will curtail the window of receptivity and increase the disposal efficacy of embryos, thus reducing the incidence of miscarriage but also increasing the likelihood of conception delay. Conversely, a disordered decidual response will increase both pregnancy as well as miscarriage rates by facilitating out-of-phase implantation (Figure 5). The reason HESCs from RPL patients fail to transit from an acute proinflammatory to an anti-inflammatory phenotype upon decidualization is unknown. However, it is reasonable to assume that the responsiveness of endometrial cells to decidualizing cues in a given cycle relates to the antecedent activation of regenerative MSCs and their programming into mature HESCs. Interesting, a preliminary study reported an inverse correlation between the abundance of clonogenic MSCs in prepregnancy endometrium and the number of previous miscarriages (133). If substantiated, this observation raises the possibility that the aberrant decidual response in RPL is caused by premature senescence. Cellular senescence is increasingly recognized as a prominent mechanism for tissue remodeling. It is defined by stable cell-cycle arrest and senescence-associated secretory phenotype, which refers to the characteristic production of various proinflammatory cytokines, chemokines, and proteases (619). The senescence-associated secretory phenotype not only promotes tissue remodeling but also prevents fibrosis in response to organ damage (620), which in the uterus is of paramount importance for future pregnancies.

Taken together, it seems more than likely that the decidual response is programmed to vary from cycle to cycle and across the reproductive years, ultimately reflecting the cumulative effects of menstruations, miscarriages, and births. Extrapolating further, this means that the human uterus is intrinsically capable of adapting to variation in embryo quality through rebalancing its receptivity and selectivity traits (Figure 5). This paradigm fits well with the epidemiology of both sporadic and recurrent miscarriages. For example, it predicts a higher incidence of sporadic miscarriages in young adolescent girls, predicated on the relative lack of cyclic recruitment of extravuterine MSCs and/or activation of resident progenitor cells, and of course in older women because of oocyte deterioration and the rapid increase in embryonic aneuploidies. It also predicts that a similar pathological pathway accounts for sporadic as well as recurrent miscarriages. Although an increasing number of losses may correlate with a lower capacity of the uterus to adapt, the cumulative success rate after several miscarriages is nevertheless predicted to be high in most RPL patients. This is unequivocally the case
as aptly demonstrated by several clinical trials reporting
that between 65% and 80% of placebo-treated subjects
will have a live-birth in the pregnancy that follows 3
previous miscarriages (621, 622). As already indicated,
the proposed paradigm also explains the apparent
trade-off between rapid conceptions and increased mis-
carriage rate (11, 623) and correctly predicts that the
attrition rate will be highest during the preclinical phase
of pregnancy (551).

Beyond the implantation stage, several additional
mechanisms are at play to ensure that a potentially failing
pregnancy is rejected in a timely fashion. For example, the
dependence of the decidual process on continuous ovarian
progesterone production during the first few weeks of
pregnancy ensures elimination of embryos perceived to
lack fitness because of insufficient hCG production. Fur-
thermore, the transition from histiotrophic nutrition of
the early conceptus to active maternal perfusion of the
placenta toward the end of the first trimester of pregnancy
serves as a robust stress test of the fetomaternal interface
because it is associated with dramatic changes in local
oxygen tension and production of free radicals (624, 625).
Arguably, this transition imposes a compulsory stress test
on the fetomaternal interface (Figure 4), ensuring that if a

Figure 5. A variable strategy for reproductive success: one size does not fit all. Preimplantation human embryos are genomically remarkably
diverse. Blastocysts can be made up of only aneuploidic blastomeres, exhibit a variable degree of cellular mosaicism or, occasionally, be perfectly
euploid. Apart from bestowing adaptability onto the species through genetic diversity, the reason for the intrinsic genomic instability in human
preimplantation embryos is not clear. However, induction of aneuploidy is associated with proteotoxic stress, metabolic overdrive, and production
of proteases, which in turn may confer increased invasiveness, as is the case for cancer cells. If so, it follows that perfect diploid blastocysts may
have high developmental potential but lack intrinsic implantation competence, not unlike murine embryos. The maternal answer to this embryonic
challenge is spontaneous decidualization, which, by being inextricably coupled to cyclic menstruation and renewal, enables the endometrium to
adapt and rebalance its receptivity vs selectivity traits. If decidualizing endometrium persistently fails to transit from an inflammatory pronidation
phenotype, implantation is unhindered, but subsequent pregnancy failure is likely for all but the most developmentally competent embryos. Lack
of decidualization or an excessive response increases the barrier function of the endometrium, leading to implantation failure and conception
delay. This system, which requires balancing of endometrial and embryonic phenotypes, works remarkably well in most women, ensuring that the
likelihood of a successful pregnancy outweighs that of a clinical miscarriage.
pregnancy is poised to fail, it is most likely to fail before 12 weeks gestation.

VI. Conclusions and Perspective

Our understanding of the pathways that control endometrial decidualization is increasing rapidly. The combination of primary human cell cultures and animal models provides a powerful system to interrogate this differentiation process, and yet translation of all of this information into new predictive tests and interventions that prevent pregnancy complications has still to materialize. Although the reasons for the sluggish progress are multifold, it is important to draw attention to 2 major weaknesses that affect most, if not all, studies on decidualization of HESCs to date. First, it is commonplace to view decidualization as a static phenotype, defined by the induction of marker genes, such as PRL or IGFBP1, at a given time point after treatment. Although this may be convenient, it ignores the fact that decidualization is a dynamic multistep process that involves transition of an acute inflammatory initiation phase to an anti-inflammatory secretory phase and finally a resolution phase, triggered either by falling progesterone levels, embryo-induced cellular stress, senescence, or a combination of these processes. As outlined in this review, the timeline that underpins these transitions critically couples endometrial receptivity with pregnancy outcome. It follows that events or interventions before implantation will have profound consequences for the subsequent trajectory and outcome of the pregnancy. This point was elegantly illustrated by experiments in Marmoset monkeys. In this primate model, the RLN receptor relaxin/insulin-like family peptide receptor 1 is induced massively in the peri-implantation phase of conceptive cycles, whereas its expression remains low or undetectable in nonconceptive cycles (626). Remarkably, postovulatory administration of RLN, which drives cAMP production in decidualizing cells, resulted in parturition 7 to 10 days earlier than in control monkeys. However, all neonates had normal birth weights and none showed signs of prematurity, highlighting the potential of peri-implantation interventions in preventing pregnancy complications and possibly even accelerating gestation (626).

The second weakness relates to the sheer plasticity of the endometrium or, to paraphrase William Potts Dewees, “the powers of the system alone.” Most adult tissues contain resident stem cells, which compensate for physiological cell attrition and enable regeneration in response to tissue damage. The human endometrium, however, is remarkable because it exhibits physiological tissue injury that leads to cyclic disposal and renewal of its superficial layer at menstruation. In this review, we have highlighted evidence that links endometrial repair and regeneration to reproductive fitness. Uterine plasticity renders it intrinsically difficult to predict the likelihood of a successful pregnancy, whether conceived spontaneously or after IVF, based on analysis of gene expression in a timed biopsy during a preceding cycle. However, markers of uterine plasticity, such as the abundance of clonogenic endometrial MSCs, may well be useful to stratify at-risk patients for interventions that increase the likelihood of successful pregnancy. Perhaps a little ironic, but nevertheless supporting our conjecture, is the increasing body of evidence showing that the mere act of taking a biopsy before an IVF treatment cycle significantly increases subsequent live-birth rates (627, 628). Whether or not recruitment or activation of endometrial MSCs account for the enhancement in uterine performance after intentional tissue injury remains to be tested. Beyond doubt, however, is that the next step-change advance in reproductive medicine will be based on strategies that target cyclic endometrial regeneration and spontaneous decidualization.

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References


5. Ramsey EM, Houston ML, Harris JW. Interactions of the trophoblast and maternal tissues in three closely related


39. Lawn AM, Wilson EW, Finn CA. The ultrastructure of


90. Schatz F, Lockwood CJ. Progestin regulation of plasminogen activator inhibitor type 1 in primary cultures of endometrial stromal and decidual cells. J Clin Endocrinol Metab. 1993;77:621–625.


102. Takano M, Lu Z, Goto T, Fusi L, et al. Transcriptional cross talk between the forkhead transcription factor forkhead box O1A and the progesterone receptor coordinates cell cycle regulation and differentiation in human endo-
134. Johansson ED, Wide L. Periovulatory levels of plasma pro-


194. Jones RL, Salamonsen LA, Findlay JK. Activin A promotes...


ceptrons (mPRα, β, and γ) localize to the endoplasmic reticulum and are not activated by progesterone. Mol Endocrinol. 2006;20:3146–3164.


320. Clemens RE, Sorescu D, Dikalova AE, et al. Nox4 is required for maintenance of the differentiated vascular...


422. Estella C, Herrr I, Atkinson SP, et al. Inhibition of histone


489. Grewal S, Carver JG, Ridley AJ, Mardon HJ. Implantation of the human embryo requires Rac1-dependent endome-


525. Koopman KA, Kopcow HD, Rybalov B, et al. Human decidual natural killer cells are a unique NK cell subset with...


