Endocrine Reviews
EARLY RELEASE:

Cyclic Decidualization of the Human Endometrium in Reproductive Health and Failure

Birgit Gellersen[1], Jan J. Brosens[2]

[1]Endokrinologikum Hamburg, 20251 Hamburg, Germany; [2]Division of Reproductive Health, Warwick Medical School, University of Warwick, Coventry CV4 7AL, United Kingdom

Key terms: human endometrium, decidualization, implantation, reproductive failure, pregnancy, stem cells, transcription, signaling

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 cyclic AMP 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.

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*) 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 two groups, the 'Nondeciduata' and the 'Deciduata': "In the Nondeciduata the fetal villi of the placenta are, at birth, simply withdrawn from the uterine

Abbreviations:

ISSN Print 0163-769X ISSN Online 1945-7189
Printed in U.S.A.
Copyright © 2014 by the Endocrine Society
Received May 29, 2014. Accepted August 14, 2014.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

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 taxon 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 peri-ovulatory period), a placenta that invades maternal blood vessels (hemochorial placentae) deeply, and give birth to only one or two 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 (QOL) and socio-economic cost, is immense. For endometriosis alone, the total annual societal cost is calculated to be approximately $65 billion in the USA (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).

While his metaphors may be dated, Dewees's views on the purpose of spontaneous decidualization and cyclic menstruation were almost prophetically precise. Only over the last few years has it become clear that decidualizing cells play an important role in recognition and elimination of developmentally impaired embryos (11, 12). Further, there is a growing realization that menstruation plays an important role in imposing cyclic recruitment 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.

I. 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, which sets out the criteria for histological dating of the endometrium (14). 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 preg-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

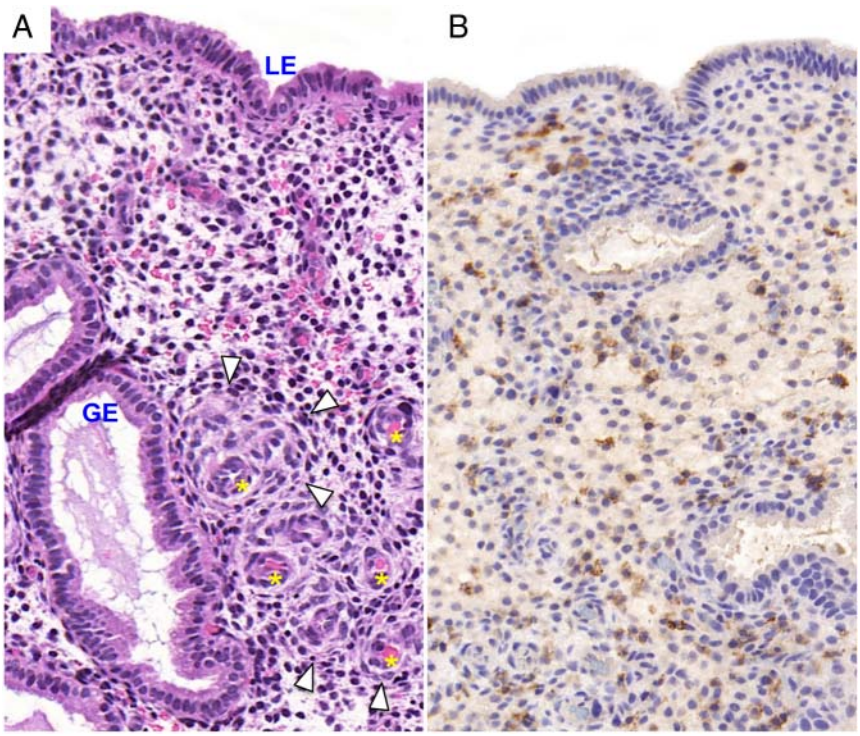
nancy. This histological appearance of the endometrium was referred to as 'predecidua' (15) (Figure 1).

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 22–23 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 2–3 days before menstrual shedding (14).

Extensive crosstalk takes place between uterine stromal and immune cells. The uterine leukocyte population is made up largely of uterine natural killer (NK) (uNK) cells, a specialized population that stains intensely for CD56 but not for CD16 antigens ($CD56^{bright}/CD16^{-ve}$) (Figure 1). After ovulation, uNK cell numbers increase dramatically and they often make up a substantial proportion (30 to 40%) of cells in the stromal compartment. The uNK cell population remains prominent in early decidua but, in the absence of pregnancy, vanishes prior to menses (16). In contrast to their circulating ($CD56^{dim}/CD16^{+}$) NK counterparts, uNK cells are not thought to function primarily as cytotoxic lymphocytes. They express killer-cell immunoglobulin-like receptors (KIR) that preferentially bind to human leukocyte antigen (HLA)-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 prior to and during trophoblast invasion (18–20).

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). uNK cells traffic in response to chemokines such as chemokine (C-X-C) motif ligand 9, CXCL10, CXCL14 and trophoblast-derived CXCL12 (19, 24). In addition, cytokines produced by resident stromal cells, including interleukin-11 (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 (11β-HSD1), an enzyme that catalyzes the conversion of inert cortisone to active cortisol (29). uNK cells are devoid of progesterone receptors (PGR) but express glucocorticoid receptors (GR) 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

Figure 1.



Spontaneous decidualization is associated with intense tissue remodeling. A, hematoxylin and eosin stained superficial endometrium on day 24 of a regular cycle. The edema of the stromal compartment underlying the luminal epithelium (LE) is regressing whereas the stromal cells surrounding the spiral arterioles (indicated by yellow asterisks) are mounting a decidual response (arrow heads), characterized by cytoplasmic expansion and rounding of cells. GE indicates glandular epithelium. B, CD56 immunostaining of a midluteal biopsy showing an abundance of uNK cells. Original magnification of A & B: × 20.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

complex and dynamic gradients of chemo-attractants 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 prior to menstruation in response to a fall in circulating progesterone (33, 34). In addition to 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 of pregnancy (35, 36). Depletion of uDCs in the mouse leads to decidualization failure in the absence or presence of an embryo, and perturbed angiogenesis (37).

B. Cellular and ultrastructural characteristics

In the proliferative phase, endometrial stromal cells have a fibroblast-like appearance with well-developed rough endoplasmic reticulum (rER) and Golgi apparatus, little cytoplasm and elongated, indented nuclei (38, 39). Decidual transformation, both in vivo and in vitro, is associated with rounding of the nucleus, increased number of nucleoli, dilatation of the rER and Golgi systems, and accumulation of glycogen and lipid droplets in the expanding cytoplasm (40). In the early- and midsecretory phase of the cycle, slender cytoplasmic processes extend into the edematous connective tissue, possibly releasing glycogen and other secretory products into the extracellular space. In the late secretory phase, decidualizing cells show pseudopodia-like extensions engulfing extracellular matrix, and the number of intracellular phagosomes and lysosomes is increased. Decidualizing stromal cells exhibit phagocytotic activity, which may contribute to the intense remodeling of the extracellular matrix (38).

Intercellular communication between endometrial stromal cells plays a critical role in establishing a functional feto-maternal interface. Cadherin-11, a mesenchymal member of the cadherin family of adhesion complex proteins, is up-regulated with decidualization, especially in stromal cells surrounding spiral arteries, and may facilitate homophilic interactions between adjacent cells as a prerequisite for the formation of gap junctions. Moreover, cadherin-11 is present on syncytiotrophoblast and extravillous cytotrophoblast and thus positioned to direct decidual-trophoblast interactions (41, 42). Gap junctions are observed between neighboring decidualizing cells (39). The gap junction protein connexin 43 (CX43) is most highly expressed in the stromal compartment during the late proliferative and early secretory phase, after which the levels diminish dramatically and are virtually undetectable by the late secretory phase of the cycle (43). Nevertheless, knockdown or pharmacological inhibition of CX43 in primary human endometrial stromal cells (HESCs) impairs decidualization (44), and ablation in mice leads to decidual failure and fetal loss (45). The relevance of gap junction-mediated communication is further supported by the finding that CX43 expression is decreased in chorionic villi and decidua from patients with recurrent early pregnancy loss (46).

Decidualization is further characterized by profound changes in the composition of the extracellular matrix (ECM). While collagens 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 proteoglycan. 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 to 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 prior to 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 midto late-secretory phase (60). A paracrine

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

target regulated by stromal cell-derived PRL is the transcription factor interferon regulatory factor 1 (IRF-1) which colocalizes with PRL-R in the glandular compartment. IRF-1 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, 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 uNK cell survival, to prevent immune rejection, and to regulate water transport across the amnion towards 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 insulin-like growth factor-binding protein-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-I, a function that is modified by its phosphorylation status. While the liver produces a single highly phosphorylated isoform, decidual cells produce primarily nonor hypo-phosphorylated 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 pre-eclampsia and IUGR. In early pregnancy, high IGFBP-1 levels appear beneficial while 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 LEFTY2, originally designated as 'endometrial bleeding-associated factor' (79, 80). Further, 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 FOXO1 and CCAAT/enhancer-binding protein C/EBPβ (81), and secretory products like wingless-type MMTV integration site family member WNT-5A (82), the inhibitor of canonical Wnt signaling, dickkopf-1 (DKK1) (83), and prokineticin-1 (PROK1) (84). These observations combined with the morphological changes suggest that decidualization is an example of mesenchymal–epithelial transition (MET). A recent study provided further support for this hypothesis by demonstrating that decidualization of murine or human stromal cells is associated with down- and up-regulation, 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 periimplantation 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 diverse cellular functions, ranging from cell cycle progression, apoptosis, and oxidative stress responses to cellular remodeling and extracellular matrix organization. Numerous protocols have been established to induce decidualization in vitro (Table 1). Commonly used protocols involve treatment of cells with (i) estradiol and progesterone or a progestin, (ii) a cAMP-inducing factor or analog, or (iii) 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 up-regulation 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 prior to menstruation. Notably, decidualization is associated with altered expression of numerous ligands, receptors, signal intermediates and down-stream transcription factors, which in turn coordinate the expression of differentiation-specific transcriptional networks. In addition, several key decidual regulators are up-regulated in differentiating HESCs at the protein but not mRNA level, including the tumor suppressor protein p53 (92) and the metastatis suppressor CD82 (KAI-1) (93). Decidualization is also associated with profound changes in the cytoskeleton, exem-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

Table 1. Deciduogenic treatments used for *in vitro* differentiation of HESCs

Treatment	Duration	Endpoint	Reference
Endometrial explants			
P4 ± E2	6–10 d	PRL secretion	(55)
P4	2–28 d	PRL secretion	(141)
Primary ESC cultures			
8-Br-cAMP	12–24 h	PRL promoter induction	(297)
8-Br-cAMP	1–3 d	PRL mRNA, IGFBP-1 mRNA	(629)
8-Br-cAMP	2 d	microarray analysis (similar results compared to P4 + E2 + EGF for 10 d)	(179)
8-Br-cAMP	24–48 h	microarray analysis	(98)
CRH	8 d	PRL secretion (enhanced by MPA)	(630)
db-cAMP	4 d	PRL secretion (enhanced by MPA)	(631)
FSH ± LH or hCG	4–6 d	PRL secretion	(143)
MPA	10–20 d	PRL mRNA, IGFBP-1 mRNA	(632)
MPA + 8-Br-cAMP	4–10 d	PRL secretion	(246)
MPA + E2 + Activin A	10 d	PRL secretion	(194)
MPA + E2 + PGE2	3 d	PRL secretion	(633)
MPA + IGF-I	28 d	PRL secretion	(634)
MPA + RLN	6 d	PRL secretion	(297)
MPA + RLN ± E2	5 d	PRL secretion	(635)
MPA or P4	20 d	PRL secretion	(636)
P4 + Cortisone + 8-Br-cAMP	4 d	PRL mRNA, IGFBP-1 mRNA	(29)
P4 + DES + EGF	25 d	PRL secretion	(637)
P4 + DHT +8-Br-cAMP	4–8 d	PRL and IGFBP-1 secretion, PRL mRNA	(103)
P4 + E2	10–15 d	PRL secretion	(638)
P4 + E2	14 d	IGFBP-1 secretion	(170)
P4 + E2 + EGF	10 d	microarray analysis (similar results compared to 8-Br-cAMP for 2 d)	(179)
P4 + E2 + IL-11	12 d	PRL secretion, IGFBP-1 secretion	(639)
Decidual explants			
P4	3–10 d	PRL secretion	(640)

plified by a decrease in α-smooth muscle actin (αSMA), 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.

D. Decidual cell transcriptome and proteome

Microarray studies confirmed that decidualization involves wholesale reprogramming of gene expression in endometrial stromal cells. Depending on the differentiation protocol and array platform used, hundreds to thousands of differentially expressed genes have been identified in undifferentiated compared to decidualizing cells (29, 97–105). For example, Takano et al reported that treatment of HESCs with a decidualizing stimulus for only 3 days significantly altered the expression of 3307 genes (102). In this study, more genes were down- than up-regulated (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, growth factor, cytokine and chemokine signaling, deposition of extracellular matrix, modulation of transcription, epigenetic patterning, and post-translational modifications, 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 up-regulated cytokines, interleukins, and their receptors in cells decidualized for 2 days compared to undifferentiated control cells. By 8 days of differentiation, only 12 transcripts remained elevated while 34 other mRNAs were now 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 CSF-2 and –3, CXCL1, IL-6, and MMP-9 (107). Garrido-Gomez et al

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

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 (108).

II. Mechanisms of decidual transformation

A. Maturation of progenitor cells

The human endometrium exhibits remarkable plasticity. Endometrial regeneration and remodeling occur after parturition, following 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 cells (EPS) and mesenchymal stem cells (MSC). 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 MSC are characterized by the expression of CD146 (also known as melanoma cell adhesion molecule or MCAM) and the receptor for platelet-derived growth factor, PDGFRβ (118). Intriguingly, mature HESCs are closely related to follicular dendritic cells, which also originate from PDGFRβ-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 MSC (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 SSEA1 (stage-specific embryonic antigen 1) was shown to enrich for an endometrial epithelial cell population from the basalis with functional features of EPS (125). The precise role of EPS and MSC in endometrial regeneration requires further clarification. For example, genetic fate-mapping in mice strongly indicated that postpartum re-epithelization of the endometrium is effected by transdifferentiation of stromal MSC (126, 127).

Menstrual blood is also an important source of MSC, designated 'endometrial regenerative cells' (ERC). ERC have a similar phenotype to endometrial and bone marrow-derived MSC, expressing CD9, CD44, CD29, CD73, CD90 and CD105, but lack the hematopoietic markers CD34, CD45 and CD133 (128, 129). ERC display extensive proliferative activity in vitro, 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, MSC isolated from the decidua of term placenta (DMSC) have been shown to migrate towards mammary tumors in a rat tumor model. DMSC do not express major histocompatibility complex (MHC) class II, are thus immunoprivileged and can be tolerated in allogeneic transplantation, suggesting that DMSC 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 MSC 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 tes-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

tosterone 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 (DHEAS) 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, tissular 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, androstenediol, testosterone but not DHEAS concentrations increase 3- to 4-fold in the secretory compared to 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 to 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).. While 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 follicle-stimulating hormone (FSH), luteinizing hormone (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, corticotropin releasing hormone (CRH) and prostaglandin 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 (157). Arguably, there are important differences in the decidual process between species and whether or not the luminal epithelium is critical in relaying deciduogenic signals in human endometrium is difficult to ascertain experimentally. Despite these limitations, a bewildering array of factors has been reported to modulate the decidual response, often – but not always - first in mouse models and then in human cell culture models.

Interleukins

Decidualizing HESCs express IL-11 in a manner that parallels PRL and IGFBP-1 secretion (158). Further, inhibition of IL-11 signaling attenuates the expression of these differentiation markers (159, 160). IL-11 and its receptor subunit IL-11Rα are localized in the decidualizing stromal cells of the midlate secretory endometrium. Moreover, in IL-11R-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 postnidation uterine environment. IL-1β up-regulates 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 cytotrophoblast

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

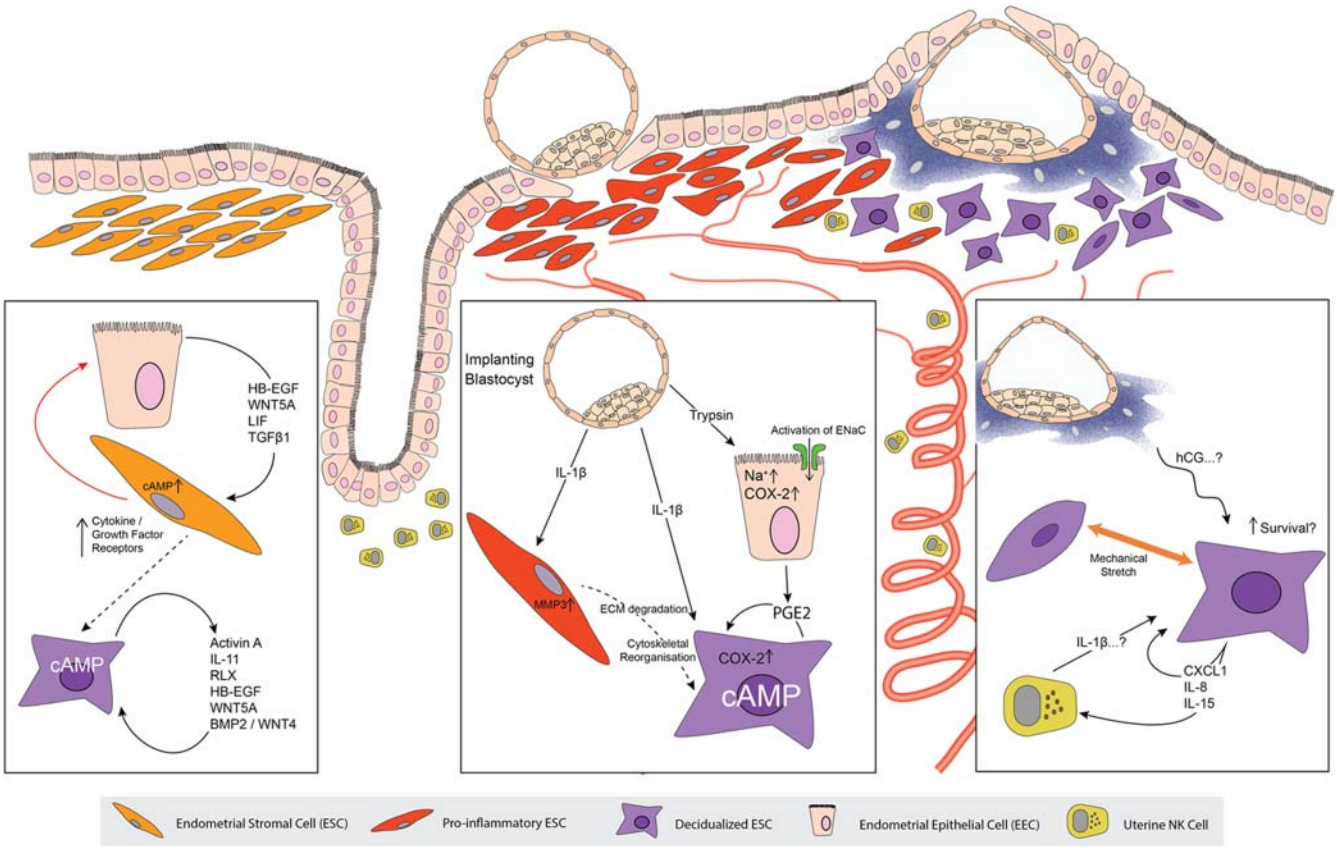
also secrete IL-1β (78, 169). Further, exposure of decidualizing HESCs to culture medium conditioned by trophoblast acutely up-regulates IL-1β transcript levels (170). It has been suggested that decidual IL-1β inhibits PRL and IGFBP-1 expression through an autocrine/paracrine mechanism (171), although 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 feto-maternal interface.

Leukemia inhibitory factor (LIF) belongs to the IL-6 type cytokines. This critical implantation cytokine is maximally expressed in the luminal epithelium just prior to 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 to 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 IVF treatment failure (185).

TGFβ superfamily and other growth factors

Deciduogenic signals induce the expression of soluble and transmembrane heparin-binding epidermal growth

Figure 2.



Multiple paracrine and autocrine regulatory inputs drive decidualization of the endometrium in a conception cycle. The spatiotemporal regulation of the decidual process is tightly controlled by morphogens, cytokines and growth factors generated by the different cellular constituents of the endometrium, including epithelial cells (left box), stromal cells, local immune cells and the vasculature. Once initiated, the decidual process is constantly evolving and transits from an acute inflammatory initiation phenotype (red) to an anti-inflammatory, highly secretory phenotype (purple). The inflammatory secretome of decidualizing stromal cells coincides with the window of receptivity during which molecular communication with the implanting embryo is established (middle box). Once the luminal epithelium is breached, decidual stromal cells surround and encapsulate the invading conceptus and mount a tailored secretory response (right box). Chemokine and cytokine gradients created by decidualizing stromal cells, uNK cells, and macrophages will direct interstitial and endovascular trophoblast migration.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

factor (HB-EGF) (186, 187) as well as at least one of its two cognate receptors, EGFR and ErbB4/HER4, 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 (ActR) 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 crosstalk 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 indispensible 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, FZD3–5), which serve as WNT receptors, as well as various WNT inhibitors (eg, DKK1, DKK2, and SFRP3) (203). Interestingly, DKK1 is also induced in response to PROK1 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 PC5/6, the proprotein convertase involved in proteolytic activation of LEFTY2 as well as vascular endothelial growth factor (VEGF) and HB-EGF precursors (205, 206). LEFTY2 and activin A are both strongly implicated in regulating the expression and activation of latent endometrial MMPs (196, 207). Interestingly, inhibition of PC5/6 or MMP expression attenuates decidualization, again emphasizing the importance of ECM remodeling in promoting HESC differentiation (206). NODAL expression in the endometrial stromal compartment increases markedly during the proliferative and early-luteal phase of the cycle. However, there is a dramatic decrease in NODAL expression during the midluteal phase of the cycle in concert with the induction of LEFTY2 (204, 208). This transition of NODAL to LEFTY2 dominance appears to be critical for implantation as either lack of uterine NODAL or overexpression of LEFTY2 severely compromise fertility in mice (209, 210). Using cells from human decidua obtained at the time of delivery, Tang and colleagues reported that overexpression of LEFTY2 pushes decidual cells to a less differentiated state and attenuates expression of decidual markers (211).

Notch signaling

The Notch signaling pathway regulates cell proliferation, differentiation and apoptosis (212). Notch is a transmembrane receptor that interacts with transmembrane ligands δ-like (DLL1, 3 and 4) or Jagged (Jag1 and 2) on neighboring cells. Upon ligand-dependent activation, serial consecutive proteolytic cleavage events lead to release of the Notch intracellular domain, which regulates transcriptional complexes in the nucleus (212). In female mice, Notch1 is increased in uterine stromal cells upon implantation or in response to artificial decidualization. Pharmacological inhibition of Notch signaling by γ-secretase inhibition impairs the decidual response and induces the expression of apoptotic mediators (213). In the human endometrium, the receptor forms Notch1–3 have been detected in epithelial and stromal cells whereas the ligands

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

DLL4 and Jag1 are predominantly localized to epithelial cells. Among target genes of activated Notch1 in HESCs are Krüppel-like factor 9 and IGFBP-1, supporting a juxtacrine role of this signaling system in the spatiotemporal control of the decidual process (214). In the baboon, Notch1 is expressed in secretory phase endometrial stromal cells around spiral arteries. Expression is greatly enhanced in the stromal compartment upon infusion of hCG to mimic pregnancy (215). Interestingly, progesterone in the presence of hCG increases the availability of cleaved Notch1 in HuFs, which refers to *h*uman *u*terine *f*ibroblasts that are isolated from decidua parietalis at the time of delivery of the placenta at term (216). Interestingly, co-immunoprecipitation experiments indicated that Notch binds PGR independently of ligand in HuFs (215), although how this interaction impacts the transcriptional activity of this nuclear receptor is not yet known.

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 CNR1 is markedly induced upon decidualization (97). In the presence of CNR1 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 (PLD1), which hydrolyzes membrane phospholipids like phosphatidylcholine. PA effects a wide range of cellular processes including cytoskeletal organization, migration, nutrient sensing, proliferation and survival (219). PLD1 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 utilized in membrane organization. Disruption of this pathway in the uterus compromises decidualization, leading to hemorrhage and embryonic demise (222)

Other local factors

Microarray studies have revealed that upon decidualization several additional factors are secreted capable of modulating ESC 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 (SSTR2), 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 growth hormone (GH) secretagogue receptor (GHS-R). Ghrelin and GHS-R 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 if these findings can be extrapolated to prepregnancy HESCs. For instance, PRL secretion from HuFs can be stimulated by placental glycoprotein hormone free α-subunit, but not hCG β-subunit (227), or 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 parathyroid hormone-like hormone (PTHLH). Knock-down 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 to the secretory phase (232). PTHLH production in cultured HESCs is under positive control of E2 (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 Alan 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

Nuclear and membrane-bound progesterone receptors

Progesterone acts by binding and activating PGR, one of the 48 members that make up the human superfamily of

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

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 two 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 PR variants are actually expressed at physiologically relevant levels in vivo (239). While 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 (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, while 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 (eg, HSP90, HSP40, HSP70 and p23) and immunophilins (eg, FKBP4 and FKBP5) (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 nongenomic 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 progesterone receptor membrane component 1 (PGRMC1) (251, 252) and various members of the progestin and adipoQ receptor (PAQR) family (253, 254). Although expressed in the human endometrium in a cycle-dependent manner (255–258), the involvement of either PGRMC1 or PAQRs in relaying progesterone actions in the endometrium remains contentious (259, 260). PGRMC1, also known as σ-2 receptor, is a cytochrome-related protein recently implicated in autophagy and clearance of damaged organelles (261, 262). It is possible 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 GRB2 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).

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, NR3A1), is essential for PGR expression (238, 268), uterine PGR continues to be expressed in ESR1 knock-out (ERKO) mice, albeit at lower levels. Further, the physiological responses to progesterone, including de-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

cidualization, are unimpaired (269). ESR1 and PGR immunostaining is prominent in proliferative epithelium and stroma. In midlate 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 progesterone 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 miRNAs 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 co-

Table 2. Genes critical to implantation and decidualization: phenotypes in female knock-out mice

Deleted gene	Phenotype[a]	Reference
Acvr1 (Alk2)	decidualization failure	(202)
Bmp2	decidualization failure	(200)
Bmpr2	decidualization failure	(641)
Bsg	implantation failure	(642)
Cdh1	implantation failure; decidualization failure	(643)
Cebpb	defective stromal cell proliferation; decidualization failure	(324, 644)
Ctnnb1	implantation failure	(645)
Dedd	decidualization failure	(646)
Dlgap5 (Hurp)	implantation failure; defective stromal cell proliferation; decidualization failure	(647)
Errfi1	implantation failure	(648)
Fkbp4 (Fkbp52)	implantation failure; decidualization failure	(278, 353)
Foxa2	implantation failure; decidualization failure	(649)
Gja1 (Cx43)	decidualization failure	(45)
Hand2	implantation failure	(650)
Hbegf	implantation failure	(189)
Hmx3	implantation failure	(651)
Hoxa10	implantation failure; defective stromal cell proliferation; decidualization failure	(277, 347)
Hoxa11	implantation failure; decidualization failure	(346)
Ihh	implantation failure	(652)
IL-11R	decidualization failure	(25, 161)
Il6st (Gp130)	implantation failure	(653)
Klf5	implantation failure; decidualization failure	(654)
Lif	implantation failure	(181, 655)
Msx1/Msx2	implantation failure	(656, 657)
Ncoa2 (Src2)	decidualization failure	(658)
Nodal	decidualization failure	(209)
Notch1	decidualization failure	(213)
Nr2f2 (COUP-TFII)	defective stromal cell proliferation; decidualization failure	(377, 379)
Nr3C3 (PGR-A)	decidualization failure	(242, 659)
Nr5a2	decidualization failure	(375)
Phb2 (REA)	decidualization failure	(280)
Prlr	implantation failure	(69)
Pten	decidual regression failure	(660)
Ptgs2 (Cox-2)	implantation failure; decidualization failure	(661)
Ptx3	implantation failure; decidualization failure	(662)
Smo	decidualization failure	(663)
Sphk1/Sphk2	decidualization failure	(222)
Src (c-Src)	decidualization failure	(435)
Trp53 (p53)	implantation failure; decidual senescence	(393, 664)
Wnt4	implantation failure; decidualization failure	(201)
Wnt7a	implantation failure	(665)

[a] Failure may be complete or partial

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

regulator 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. Knock-down of REA by siRNA sensitizes HESCs to deciduogenic 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 in order 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.

2. Cyclic AMP signaling

Regulation of intracellular cAMP levels

cAMP is a ubiquitous second messenger produced upon binding of extracellular ligands to G_s protein-coupled receptors. Subsequent activation of membrane-bound adenylyl cyclase leads to generation of cAMP from adenosine triphosphate (ATP). A major target for cAMP is protein kinase A (PKA), a holoenzyme composed of two regulatory (R) and two 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 (CBP; p300) with inherent histone acetyltransferase activity modulates chromatin conformation and thus facilitates transcription initiation (288).

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

Intracellular levels of cAMP, and of cyclic guanosine monophosphate (cGMP), depend not only on the rate of production but also the rate of degradation 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 C subunit of PKA, thus reducing target protein activation, and up-regulation of R subunit, which in turn leads to increased cAMP scavenging (284).

cAMP signaling in decidualizing endometrial cells

Adenylyl cyclase activity in the endometrium is higher than in the myometrium, corpus luteum, or Fallopian tube (292). Furthermore, PGE2-stimulated adenylyl 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 to 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 down-regulation of the regulatory PKA subunit RIα. 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).

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

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. siRNA-mediated knock-down of EPAC1 or EPAC2 blocks differentiation of HESCs (301). Apart from Rap2, calreticulin (CRT), a Ca^{2+}-binding storage protein and molecular chaperone in the ER, 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, ICER (inducible cAMP early repressor) is up-regulated 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

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 and both related to the organization of the human *PRL* gene. First it was shown that decidualizing HESCs, as well as human lymphocytes, utilize a different promoter in the *PRL* gene when compared to 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, thyrotropin-releasing hormone (TRH), or the transcription factor PIT-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 a 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 ETS1 and a number of ubiquitous transcription regulators, such as CBP/p300 and Yin Yang 1 (YY1) (313).

At a functional level, MER20s from 20 genes, including *PRL*, *WNT4*, *WNT5A*, *INHA* (inhibin alpha chain), and *HSD11B1*, were shown to confer responsiveness to cAMP and progestin 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 coopted the cAMP and progesterone signaling pathways to drive expression of decidua-specific genes (313). Depending on the pro-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

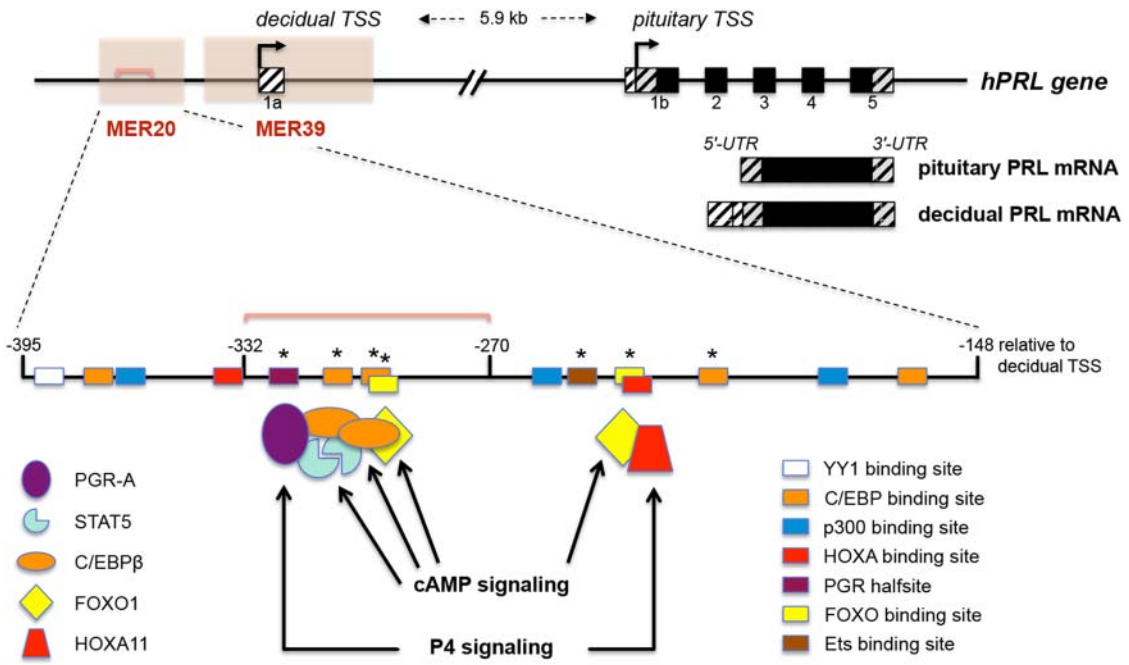
moter context and state of differentiation, a host of transcription factors in addition to the core initiators are involved in fine-tuning decidual gene networks.

Endometrial PRL expression is not shared by all placental mammals, as it is not evident in rabbits, pigs, dogs or armadillos (315). It has been demonstrated in primates, mice and elephants and involves, as in the human, the utilization 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.

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 isoforms, is a relatively early event in HESCs treated with a cAMP analog (318). Further, the DNA binding activity of C/EBPβ increases markedly in response to cAMP signaling in HESCs, which is mediated by activation of nicotinamide adenine dinucleotide (NAD) phosphate (NADPH) oxidase complexes and a surge in endogenous reactive oxygen species (ROS) (309). Notably, activation of the NADPH oxidase complex and transient redox signaling is not specific to decidualizing HESCs but

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 utilized which is located 5.9 kilobase (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 transcriptional start site (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.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

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 G_1-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.

FOXO transcription factors

The FOXO subfamily of Forkhead transcription factors consists of three members in mammals, FOXO1, FOXO3a, and FOXO4. Functionally, they represent a hybrid subclass in that they not only act 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 (PI3K)/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 post-translational 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 upregulated 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 (BMP and activin membrane-bound inhibitor), 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, 337, 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 indispensible 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 proapoptotic Bcl-2 family member BIM, 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.

HOX proteins

The *HOX* genes are developmental genes that cluster in four 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 paralogues 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 *Hox13* 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

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

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). Knock-down of *HOXA10* attenuates some (eg, PRL, IGFBP-1) but enhances other (eg, IL-11, 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 aforementioned, this immunophilin is indispensible 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 prior to and during the process of differentiation, a response counterbalanced by the antiproliferative actions of FOXO1 (100). On the MER20 enhancer of the decidual *PRL* promoter, HOXA11 acts as a transcriptional repressor but is converted to a strong activator in the presence of FOXO1 (354).

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 11βHSD1, 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 feto-maternal 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 PCBP1 that targets AR transcripts (361). In agreement, studies in primates have shown that progestins 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 down-regulated 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 up-regulation 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 progestin 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 cortisone. The rationale was that induction of endogenous 11βHSD1 activity in differentiating HESCs would convert cortisone 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 sup-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

presses 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 tumor necrosis factor (TNF) receptor superfamily, apoptosis mediators, oxidative stress defenses, and DNA repair enzymes. Thus, the critical dependency of the feto-maternal interface on sustained progesterone signaling is mediated at least in part by (i) PGR-mediated repression of factors involved in tissue destruction, and (ii) reprogramming of pathways activated by growth factors, cytokines and other cues that control decidual gene networks (103).

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, LRH-1 (liver receptor homolog-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 only possible 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-TFII 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 up-regulated 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 perturbs 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 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 up-regulated 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 prior to implantation (384).

Signal Transducers and Activators of Transcription (STATs)

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, one of seven mammalian STAT isoforms, is the downstream regulator of IL-11 signaling in decidualizing HESCs. Levels of STAT3 as well as

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

SOCS3 are elevated in decidualized cells, with the former being progesterone- 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 immunotolerance to the fetal allograft (388).

The tumor suppressors p53 and 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 up-regulated 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 2–4 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 which 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. Further, 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 ROS (400). Remarkably, p53 shares many functional features and target genes with FOXO1, rendering them partners to survey genomic integrity in the decidua (401).

While p53 in decidualizing HESCs is exquisitely cAMP-responsive and regulated at the protein level, the transcription factor promyelocytic leukemia zinc finger (PLZF; also known as ZF145 or ZBTB16) is solely induced by progesterone or glucocorticoids at transcriptional levels (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 SMRT (silencing mediator for retinoid and thyroid-hormone receptors), N-CoR (nuclear receptor corepressor), Sin3 and HDACI (histone deacetylase I) (404, 405). It exerts antiproliferative actions, eg, by suppressing the cyclin A promoter, and can confer resistance to apoptosis by inhibiting expression of BID, 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 up-regulation of PLZF, in turn, promotes survival. FOXO1, as the third player, is positioned at the crossroads of apoptosis and survival, being up-regulated 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).

Other transcriptional regulators

The composite transcription factor activating protein 1 (AP-1) serves as a major regulator of cell proliferation as

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

it integrates various mitogenic signals. The AP-1 complex is composed of homo- or heterodimers of Jun, Fos and ATF proteins, members of the bZIP family of transcription factors. The Jun family consists of c-Jun, JunD and JunB, while 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 (E twenty-six) transcription factor superfamily that is defined by a highly conserved eighty-five 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 four 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 while Sp3 expression decreases in HESCs upon decidualization (413). The expression of several decidua-specific genes, such as *F3* (encoding TF), *PAI1*, and *IGFBP1*, has been shown to be regulated by the cellular Sp1/Sp3 ratio (414).

Another factor gaining prominence in the decidual process is GATA2. 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, while GATA2 is not induced upon decidualization of human ESCs, 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 (DNMTs) (420). For example, the histone methyltransferase enhancer of Zeste homologue 2 (EZH2) is gradually lost upon decidualization of HESCs, which in turn results in declining levels of trimethylated lysine 27 of histone 3 (H3K27me3) at the proximal promoters of key decidual marker genes such as *PRL* and *IGFBP1*. Further, loss of H3K27me3 at these sides is associated with a reciprocal enrichment in acetylation of the same lysine residue, indicating active remodeling from a repressive to a transcriptionally permissive chromatin. Genome-wide 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 (TSA), a histone deacetylase inhibitor, enhances *PRL* and *IGFBP1* expression in a dose-dependent fashion in differentiating HESCs (421). Furthermore, TSA 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 methylation inhibitor 5-Aza-2′-deoxycytidine is sufficient to trigger expression of a subset of decidual genes involved in cell adhesion, ECM remodeling and cytoskeletal organization (423). 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). Further, 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 nucleo-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

somes. 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 7,034 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 'cistrome'. Interestingly, a comparison of the PGR cistromes in breast cancer and leiomyoma cells identified only 1,035 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. Posttranslational modifications and convergence of signaling pathways

Activation of the cAMP and progesterone signaling pathways has profound ramification for signal transduction through other pathways. On the one hand, this is effected 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 progestin inhibits the expression of several genes encoding for components upstream of JNK while simultaneously up-regulating MAPK phosphatase 1 (MKP1). 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 as both kinases are not only closely related but also controlled by identical upstream regulators: PI3K, PDK1 (phosphoinositide-dependent kinase 1) and mTORC2 (mammalian target of rapamycin complex 2) (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). As 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. Beside phosphorylation, the activity of transcription factors is further regulated by a host of other post-translational modifications, including glycosylation, ubiquitination, nitrosylation, methylation, acetylation, and sumoylation. Protein post-translational 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, an E2 conjugating enzyme (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

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

PIAS1 (protein inhibitor of activated STAT1) and up-regulation 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 progestin treatment without a need to simultaneously activate the cAMP pathway (438).

III. 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 (i) the positioning of the embryo near the fundus of the uterus, (ii) absorption of uterine fluid, luminal 'closure' and apposition of the blastocyst on the endometrial surface epithelium, (iii) stable adherence to the apical surface of luminal epithelial cells, (iv) penetration through the luminal epithelium and its basal lamina, and, finally, (v) 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 prereceptive (days 1–3) to a receptive (day 4) and finally a refractory phase (day 5 onwards) (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 to 7 days after the LH surge, free-floating embryos can 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 ten previous pregnancies resulting in nine 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 demonstrated that 84% of conceptions can be detected between 8 to 10 days after ovulation (452). 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 (ART). 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 two genes were common to all 6 studies, *SPP1* (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 relative 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). While 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/nonreceptive) originated from observations in polytocous species, foremost mice. Here, reproductive success is based on rapid breeding cycles, synchronized implantation of multiple embryos, large litter sizes and selection of pups after

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

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. While 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 foremost 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 opposing views have been referred to as "cooperation and conflict in the placental bed" (10). Whether or not the embryo is the aggressor has been a matter of considerable historical debate. In 1910, Ernst Gräfenberg, a German gynecologist and scientist, stated that in previous decades the ovum had been viewed as "a passive object around which maternal tissues spread like a protective cover", whereas now the view had changed dramatically: "Far from being a helpless germ in need of protection, the ovum is a cheeky intruder [ein frecher Eindringling] that eats its way deep into the uterine mucosa" (10, 484). By the mid 20th century, this view of the endometrium as "receptive and defensive" and the trophoblast as "invasive and proliferative" became widely established (485, 486). A PubMed search for the keywords 'human trophoblast AND invasion' currently yields more than 1600 articles.

Recent evidence, however, has revived the original view that maternal cells actively encapsulate the conceptus (479), without necessarily questioning the invasive nature

Table 3. Of mice and men: a comparison between embryonic and decidual characteristics

	Mouse	Human
Embryonic characteristics		
Dormancy / diapause:	Yes	No
Chromosomal instability:	Rare	Very prevalent
No. of implantation sites:	± 10	1–2
Invasiveness:	Low	High
Hemochorial placenta:	Shallow	Deep
Maternal decidualization		
Trigger:	Implantation	Endocrine cues
Progesterone dependency:	Yes	Yes
cAMP dependency:	No	Yes
Cell polyploidization:	Yes	No
Terminal differentiation:	Yes	No
Menstruation:	No	Yes
Cyclic regeneration:	No	Yes

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

of the trophoblast. Decidualized endometrial stromal cells produce a rich repertoire of MMPs (487), are intrinsically motile and have an invasive capacity that is comparable, if not greater, than that of trophoblast (488). By placing human or mouse blastocysts on a monolayer of decidualized HESCs, Grewal et al noticed that stromal cell motility was obligatory for blastocyst implantation and trophoblast outgrowth. Time-lapse recordings revealed that the stromal cells moved around the embryo to accommodate its expansion (489, 490). Decidualized HESCs have been shown to provide a more favorable matrix for trophoblast expansion than do undifferentiated cells (167). EMILIN1, a connective tissue glycoprotein associated with elastic fibers, is produced by the decidua. Extravillous trophoblast cells migrate towards EMILIN1 in a process termed haptotactic directional migration, which involves integrin signaling (491). Trophoblast signals, especially PDGF-AA, in turn trigger chemotactic and invasive migration of endometrial stromal cells (95, 107, 492, 493). Furthermore, imaging studies have shown that decidualizing HESCs are programmed to migrate towards implantation-competent blastocysts (494); and transcriptome analysis of trophectoderm from human blastocysts and matched endometrial biopsies revealed that secretion of embryo-derived PDGF-AA coincides with increased expression of its receptor, PDGF-Rα, in endometrial cells (495). The ultrastructure of the early feto-maternal interface has been assessed in cocultures of decidual explants with chorionic villi (496). Within 96 hours, invaded extravillous trophoblast was visible. Extensive desmosomal cell-cell contacts were observed between cytotrophoblast and decidual cells. Notably, decidual cells showed no signs of degeneration or apoptosis, indicating that the invasion process is tightly controlled to prevent damage to the feto-maternal interface (496). Taken together, it is increasingly apparent that decidualizing cells in vivo not only tolerate implantation but actively engage in the process by encapsulating the blastocyst (167, 479, 488, 497).

C. Embryo selection at implantation

The term embryo 'selection' was coined to describe the observation that endometrial cells respond to individual preimplantation embryos in a manner that either supports further development or facilitates early rejection (12, 480). This ability to tailor a response to individual embryos is not confined to human endometrium. In fact, the notion that the endometrium serves as a biosensor of embryos originated from livestock experiments (498). In ruminants, ART and somatic cell nuclear transfer (SCNT) are associated with developmental abnormalities, including large offspring syndrome (499). Microarray analyses in the bovine have shown that endometrial gene expression in pregnancy will differ, depending on the origins (eg, SCNT, IVF, artificial insemination) and developmental potential of the implanted embryo (498). Furthermore, next-generation sequencing revealed a dramatic up-regulation of essential implantation and metabolic genes in murine uteri transiently exposed to spent culture medium of developmentally competent human embryos. By contrast, this response was entirely absent following exposure to culture medium 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 human embryos. Teklenburg and colleagues employed a human coculture model, consisting of single hatched blastocysts cultured on 50,000 decidualizing HESCs, to identify key factors involved in the embryo-maternal cross-talk. The coculture supernatants were collected after 3 days and assayed for 14 implantation cytokines, growth factors and chemokines. Expression levels were then compared to the levels produced by decidualizing cells in the absence of an embryo. Rather unexpectedly, the presence of a normally developing human blastocyst had no measurable 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 characterized by selective inhibition of several interleukins, CCL11, and HB-EGF secretion. Repeat coculture experiments with undifferentiated HESCs yielded no response irrespective of embryo quality (12). In a follow-up study, these investigators combined a modified wound-healing assay with time-lapse microscopy and found that decidualizing HESCs selectively migrate towards 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 crosstalk (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

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

of HESCs, in parallel with the expanding ER 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 (ENaC) present on the apical border of luminal endometrial epithelial cells (EECs) (480, 501). This in turns leads to an inward current, membrane depolarization, and Ca^{2+} entry into EECs through voltage-gated L-type Ca^{2+} channels. The resultant Ca^{2+} transients then phosphorylate and activate the transcription factor CREB, which in turn leads to up-regulation of COX-2 activity and PGE2 release (501) (Figure 2). Importantly, the Ca^{2+} 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 Ca^{2+} 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 foremost serve to limit maternal investment in invasive but developmentally compromised human embryos.

D. Immunomodulation and maternal tolerance

The Nobel laureate Sir Peter Brian Medawar is credited widely for articulating the paradoxical immunological relationship that exists between mother and fetus in pregnancy (502). 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 (APCs) 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 Th1 cell- and cytotoxic 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 down-regulation 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 CCL5 when compared to matched perivascular MSCs, irrespective of whether the cells are decidualized or not (Murakami K. & Brosens J.J., unpublished observations).

Additional mechanisms have been implicated in T cell tolerance towards 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 to 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 re-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

stores 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 feto-maternal 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 (FasL), which induces apoptosis of activated T cells. Remarkably, decidualizing cells also express Fas (also termed CD95 or APO-1), the cognate cell surface receptor for FasL, but auto-activation of the death-receptor signaling pathway is prevented by simultaneous induction of c-FLIP, 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 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 wellbeing 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 extravillous trophoblast (536). At this stage, macrophages make up approximately 20%–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 two 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 trophoblast (544). Experimental evidence suggests that decidual macrophages inhibit NK cell cytotoxicity as well as cooperate with NK cells to induce immunosuppressing regulatory T cells (Tregs) (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 Tregs (547, 548).

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

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

production around 6–8 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 prior to 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 prior to 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 cytoplasmic IκB levels, thus promoting nuclear NF-κB accumulation, activation of a proinflammatory gene network, COX-2 induction and PGF2α production (564, 565). As aforestated, 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 prior to 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 was the first to argue that menstruation is nothing more than a nonadaptive consequence of uterine evolution (567, 568). This widely promulgated concept, which infers that menstruation is merely a by-product of spontaneous decidualization that serves no purpose other than facilitating the next reproductive cycle (7), predates the realization that cyclic scar-free repair of the endometrium involves activation and recruitment of epithelial and mesenchymal progenitor cells. As aforementioned, apart from resident tissue MSCs, the bone marrow is a major extrauterine source of cells that make up the endometrium in both humans and mice (116, 569–573). Murine transplantation studies have shown that bone marrow cells that constitutively express green fluorescent protein are rapidly incorporated in the endometrium and constitute a major, long-term source of nonhematopoietic endome-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

trial cells (117). The flux of bone marrow-derived cells to the uterus increases in response to uterine trauma and inflammation (574), and contributes primarily to the stromal compartment and, to a lesser extent, to glandular and luminal epithelium (117). Arguably, an ability to populate, expand or adapt the uterine stem cell niche postnatally – for example at menarche – may be essential not only for tissue repair following menstruation, miscarriage or parturition but also underpin the developmental plasticity needed to accommodate deep trophoblast invasion. In this respect, it is noteworthy that 90% of all menstrual cycles in young adolescent girls are anovulatory (575), *de facto* ensuring that tissue repair – and presumably stem cell mobilization – always precedes pregnancy (576).

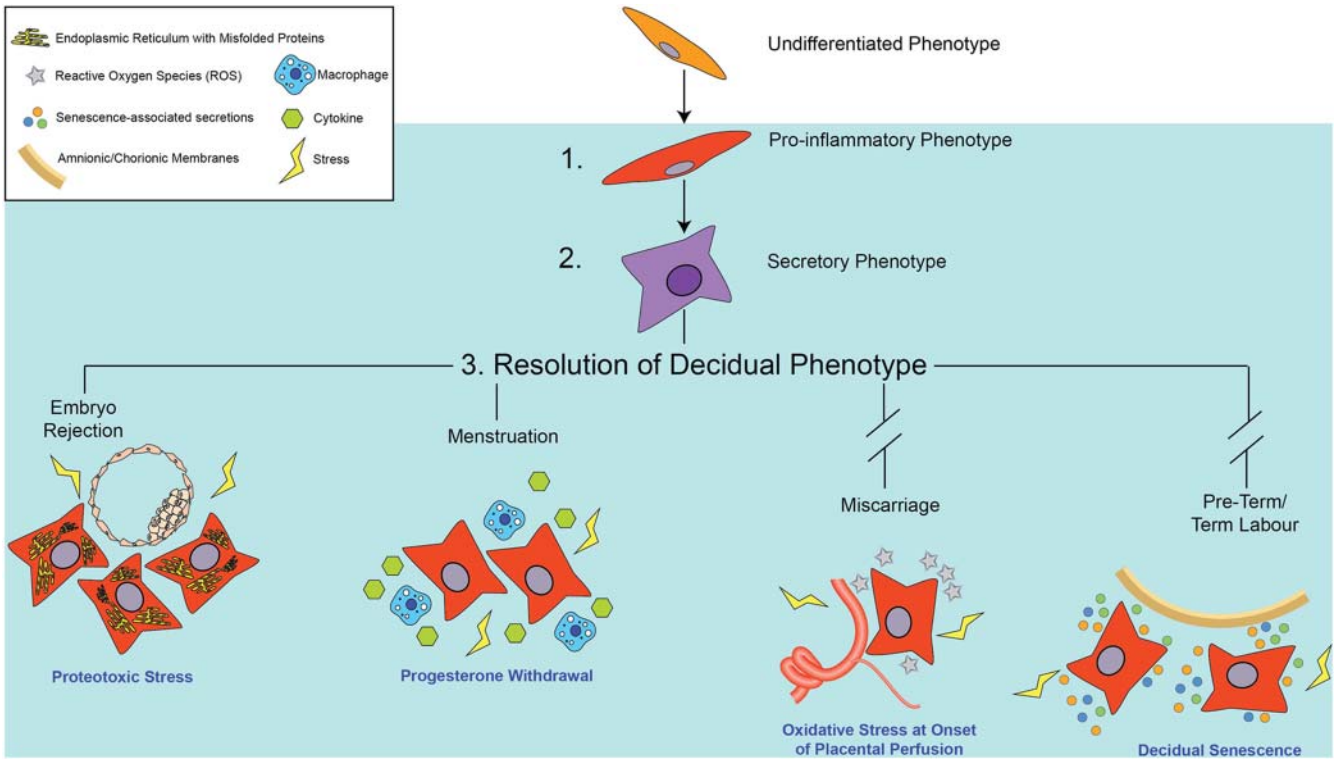
The actual regenerative process in the endometrium is far from understood and profoundly understudied. A rapid and crucial step is re-epithelialization and areas of active shedding and repair are found in close proximity in menstrual endometrium (577). Based on scanning electron microscopy (EM), glandular 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 MET 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 coexpress epithelial (pan-cytokeratin) and stromal (vimentin) cell markers, which then migrate to the regenerative zone near the lumen as the repair process unfolds (127).

IV. 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

Figure 4.



Decidualization: a dynamic pathway leading to inevitable tissue destruction. Rather than being static, decidual cells are programmed to transit through distinct phenotypic stages. The initial decidual response is characterized by expression of proinflammatory factors (1), implicating in eliciingt a receptive endometrial phenotype. This is followed by a profound anti-inflammatory response associated with a full secretory phenotype and an ability to mount a tailored response to signals from high- as well as low-quality embryos (2). The defining feature of the decidua is its transient nature and resolution is invariably associated with reactivation of inflammatory mediators (3), leading to influx of immune cells and tissue breakdown. Various signals can trigger resolution of the decidual phenotype, including proteotoxic stress triggered by developmentally compromised embryos; corpus luteum failure leading to progesterone withdrawal and menstruation; excessive oxidative stress associated with the onset of placental perfusion; and possibly cellular senescence, implicated in the onset of labor.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

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 following 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). While 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%–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 while 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 impact on the cellular identity of transplanted stromal cells and, hence, their responsiveness 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, 2,430 genes and 42,248 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 to 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, 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 as endometriosis is also associated with altered gene expression in undifferentiated primary cultures as well as refractory responses to other differentiation signals, including cAMP

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

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 as 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 following 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 impact on 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 seven recognized pregnancies end in miscarriage during the first trimester and 1%–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 (LBW) (610, 611). Furthermore, a recent meta-analysis concluded that miscarriage is associated with a greater risk of subsequent coronary heart disease (CHD) (612).

Management of recurrent pregnancy loss (RPL) is particularly challenging. The American Society for Reproductive Medicine defines RPL as two or more consecutive pregnancy losses whereas the European Society for Human Reproduction and Embryology has adopted a definition of three 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.

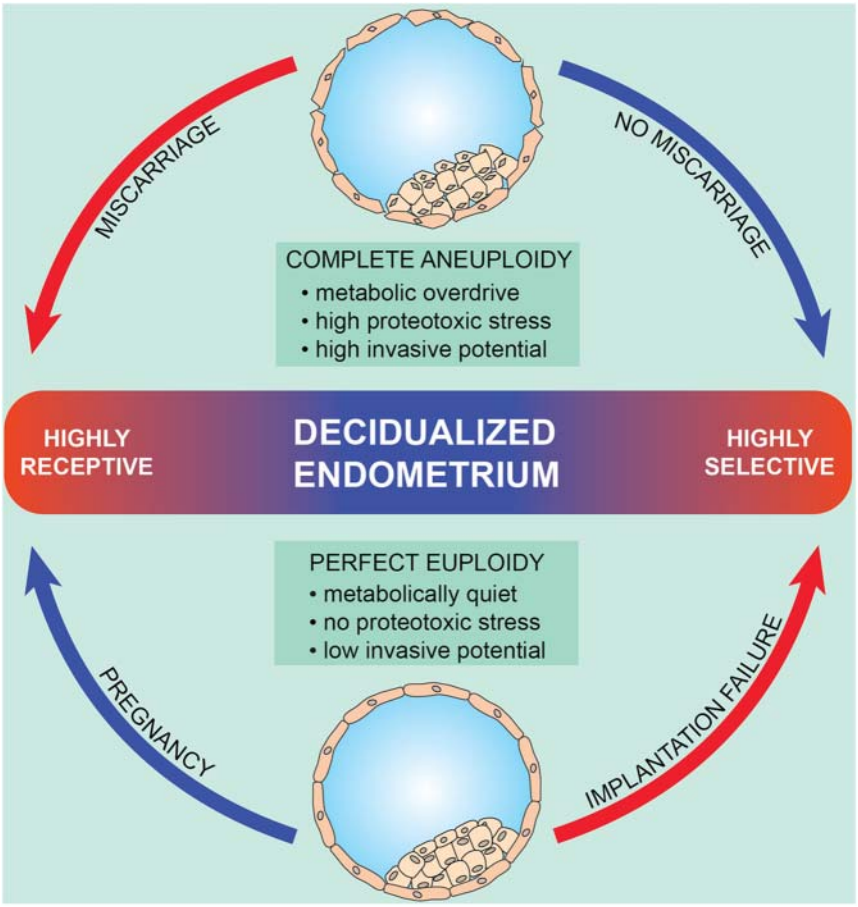
The emerging concept of active selection of human embryos at implantation provides a new ontological dimension to early pregnancy loss. In order to sense, support or reject implantation, decidualizing cells surrounding the conceptus must expand their ER and be fully secretory (555), a phenotype that 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 interleukins, chemokines, C-reactive protein (CRP) and other inflammatory mediators. Down-regulation 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

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

IL-33 secretion is 10-fold higher on day 4 compared to day 10 of decidualization; but strikingly, a reversed pattern was observed in 15 RPL cultures. Further, the induction of the anti-inflammatory decoy receptor sST2 upon 10 days of differentiation was significantly blunted in RPL compared to 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 prior to 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 why 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 deciduogenic cues in a given cycle relates to the anteceded 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 se-

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.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

cretory phenotype (SASP), which refers to the characteristic production of various proinflammatory cytokines, chemokines, and proteases (619). SASP 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 extrauterine 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. While 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 to 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 miscarriage 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. Further, the transition from histiotrophic nutrition of the early conceptus to active maternal perfusion of the placenta towards the end of the first trimester of pregnancy serves as a robust stress test of the feto-maternal interface as 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 feto-maternal interface (Figure 4), ensuring that if a pregnancy is poised to fail, it is most likely to fail before 12 weeks gestation.

V. 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 this information into new predictive tests and interventions that prevent pregnancy complications has still to materialize. While the reasons for the sluggish progress are multifold, it is important to draw attention to two 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 following treatment. While 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 time-line that underpins these transitions critically couples endometrial receptivity with pregnancy outcome. It follows that events or interventions prior to 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 RXFP1 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 since 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,

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

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 prior to 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 following 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.

Acknowledgments

We wish to thank Emma Lucas for her excellent assistance with the preparation of the manuscript, especially with the figures. This work was supported by the Biomedical Research Unit in Reproductive Health, a joint initiative between the University Hospitals Coventry and Warwickshire NHS Trust and Warwick Medical School.

Address requests for reprints to: Jan J. Brosens, MD, PhD, Division of Reproductive Health, Warwick Medical School, University of Warwick, Coventry CV4 7AL, United Kingdom, Phone: +44 2476 968704, e-mail: J.J.Brosens@warwick.ac.uk

Disclosure Statement: The authors have nothing to disclose

This work was supported by the Biomedical Research Unit in Reproductive Health, a joint initiative between the University Hospitals Coventry and Warwickshire NHS Trust and Warwick Medical School.

References

1. **Dunn PM.** William Potts Dewees (1768–1841) of Pennsylvania: pioneer of perinatal medicine in America. *Arch Dis Child Fetal Neonatal Ed*. 1996;75:F69–70.
2. **Dewees WP.** 1826 A Treatise on the Diseases of Females. Philadelphia: H.C. Carey, I. Lea
3. **Johnson GA, Burghardt RC, Joyce MM, Spencer TE, Bazer FW, Pfarrer C, Gray CA.** Osteopontin expression in uterine stroma indicates a decidualization-like differentiation during ovine pregnancy. *Biol Reprod*. 2003;68:1951–1958.
4. **Huxley TH.** 1898 A Manual of the Anatomy of Vertebrate Animals. New York: D. Appleton and Company
5. **Ramsey EM, Houston ML, Harris JW.** Interactions of the trophoblast and maternal tissues in three closely related primate species. *Am J Obstet Gynecol*. 1976;124:647–652.
6. **Brosens JJ, Parker MG, McIndoe A, Pijnenborg R, Brosens IA.** A role for menstruation in preconditioning the uterus for successful pregnancy. *Am J Obstet Gynecol* 2009;200:615.e001–e006.
7. **Emera D, Romero R, Wagner G.** The evolution of menstruation: a new model for genetic assimilation: explaining molecular origins of maternal responses to fetal invasiveness. *Bioessays*. 2012;34:26–35.
8. **Kliman HJ.** Uteroplacental blood flow. The story of decidualization, menstruation, and trophoblast invasion. *Am J Pathol*. 2000;157:1759–1768.
9. **Simoens S, Dunselman G, Dirksen C, Hummelshoj L, Bokor A, Brandes I, Brodszky V, Canis M, Colombo GL, DeLeire T, Falcone T, Graham B, Halis G, Horne A, Kanj O, Kjer JJ, Kristensen J, Lebovic D, Mueller M, Vigano P, Wullschleger M, D'Hooghe T.** The burden of endometriosis: costs and quality of life of women with endometriosis and treated in referral centres. *Hum Reprod*. 2012;27:1292–1299.
10. **Haig D.** Fertile soil or no man's land: cooperation and conflict in the placental bed. In: Pijnenborg R, Brosens I, Romero R eds. Placental Bed Disorders – Basic Science and its Translation to Obstetrics. Cambridge: Cambridge University Press 2010;165–173.
11. **Salker M, Teklenburg G, Molokhia M, Lavery S, Trew G, Aojanepong T, Mardon HJ, Lokugamage AU, Rai R, Landles C, Roelen BA, Quenby S, Kuijk EW, Kavelaars A, Heijnen CJ, Regan L, Macklon NS, Brosens JJ.** Natural selection of human embryos: impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss. *PLoS ONE*. 2010;5:e10287.
12. **Teklenburg G, Salker M, Molokhia M, Lavery S, Trew G, Aojanepong T, Mardon HJ, Lokugamage AU, Rai R, Landles C, Roelen BA, Quenby S, Kuijk EW, Kavelaars A, Heijnen CJ, Regan L, Brosens JJ, Macklon NS.** Natural selection of human embryos: decidualizing endometrial stromal cells serve as sensors of embryo quality upon implantation. *PLoS ONE*. 2010;5:e10258.
13. **Lucas ES, Salker MS, Brosens JJ.** Uterine plasticity and reproductive fitness. *Reprod Biomed Online*. 2013;27:506–514.
14. **Noyes RW, Hertig AT, Rock J.** Dating the endometrial biopsy. *Fertil Steril*. 1950;1:3–25.
15. **Rock J, Bartlett MK.** Biopsy studies of human endometrium: criteria of dating and information about amenorrhea, menorrhagia, and time of ovulation. *J Am Med Assoc*. 1937;108:2022–2028.
16. **Croy BA.** Uterine natural killer cells: a specialized differentiation regulated by ovarian hormones. *Immunol Rev*. 2006;214:161–185.
17. **Chazara O, Xiong S, Moffett A.** Maternal KIR and fetal HLA-C: a fine balance. *J Leukoc Biol*. 2011;90:703–716.
18. **Ashkar AA, Black GP, Wei Q, He H, Liang L, Head JR, Croy BA.** Assessment of requirements for IL-15 and IFN regulatory factors in uterine NK cell differentiation and function during pregnancy. *J Immunol*. 2003;171:2937–2944.
19. **van den Heuvel MJ, Chantakru S, Xuemei X, Evans SS, Tekpetey F, Mote PA, Clarke CL, Croy BA.** Trafficking of circulating pro-NK cells to the decidualizing uterus: regulatory mechanisms in the mouse and human. *Immunol Invest*. 2005;34:273–293.
20. **Xiong S, Sharkey AM, Kennedy PR, Gardner L, Farrell LE, Chazara O, Bauer J, Hiby SE, Colucci F, Moffett A.** Ma-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

ternal uterine NK cell-activating receptor KIR2DS1 enhances placentation. *J Clin Invest*. 2013;123:4264–4272.
21. **Quenby S, Nik H, Innes B, Lash G, Turner M, Drury J, Bulmer J.** Uterine natural killer cells and angiogenesis in recurrent reproductive failure. *Hum Reprod*. 2009;24:45–54.
22. **Clifford K, Flanagan AM, Regan L.** Endometrial $CD56^+$ natural killer cells in women with recurrent miscarriage: a histomorphometric study. *Hum Reprod*. 1999;14:2727–2730.
23. **Tang AW, Alfirevic Z, Turner MA, Drury JA, Small R, Quenby S.** A feasibility trial of screening women with idiopathic recurrent miscarriage for high uterine natural killer cell density and randomizing to prednisolone or placebo when pregnant. *Hum Reprod*. 2013;28:1743–1752.
24. **Mokhtar NM, Cheng CW, Cook E, Bielby H, Smith SK, Charnock-Jones DS.** Progestin regulates chemokine (C-X-C motif) ligand 14 transcript level in human endometrium. *Mol Hum Reprod*. 2010;16:170–177.
25. **Ain R, Trinh ML, Soares MJ.** Interleukin-11 signaling is required for the differentiation of natural killer cells at the maternal-fetal interface. *Dev Dyn*. 2004;231:700–708.
26. **Godbole G, Modi D.** Regulation of decidualization, interleukin-11 and interleukin-15 by homeobox A 10 in endometrial stromal cells. *J Reprod Immunol*. 2010;85:130–139.
27. **Salker MS, Nautiyal J, Steel JH, Webster Z, Sucurovic S, Nicou M, Singh Y, Lucas ES, Murakami K, Chan YW, James S, Abdallah Y, Christian M, Croy BA, Mulac-Jericevic B, Quenby S, Brosens JJ.** Disordered IL-33/ST2 activation in decidualizing stromal cells prolongs uterine receptivity in women with recurrent pregnancy loss. *PLoS ONE*. 2012;7:e52252.
28. **Kitaya K, Yasuda J, Yagi I, Tada Y, Fushiki S, Honjo H.** IL-15 expression at human endometrium and decidua. *Biol Reprod*. 2000;63:683–687.
29. **Kuroda K, Venkatakrishnan R, Salker MS, Lucas ES, Shaheen F, Kuroda M, Blanks A, Christian M, Quenby S, Brosens JJ.** Induction of 11β-HSD 1 and activation of distinct mineralocorticoid receptor- and glucocorticoid receptor-dependent gene networks in decidualizing human endometrial stromal cells. *Mol Endocrinol*. 2013;27:192–202.
30. **Guo W, Li P, Zhao G, Fan H, Hu Y, Hou Y.** Glucocorticoid receptor mediates the effect of progesterone on uterine natural killer cells. *Am J Reprod Immunol*. 2012;67:463–473.
31. **Quenby S, Kalumbi C, Bates M, Farquharson R, Vince G.** Prednisolone reduces preconceptual endometrial natural killer cells in women with recurrent miscarriage. *Fertil Steril*. 2005;84:980–984.
32. **Russell P, Sacks G, Tremellen K, Gee A.** The distribution of immune cells and macrophages in the endometrium of women with recurrent reproductive failure. III: Further observations and reference ranges. *Pathology*. 2013;45:393–401.
33. **Salamonsen LA, Zhang J, Brasted M.** Leukocyte networks and human endometrial remodelling. *J Reprod Immunol*. 2002;57:95–108.
34. **Thiruchelvam U, Dransfield I, Saunders PT, Critchley HO.** The importance of the macrophage within the human endometrium. *J Leukoc Biol*. 2013;93:217–225.
35. **King A.** Uterine leukocytes and decidualization. *Hum Reprod Update*. 2000;6:28–36.
36. **Rieger L, Honig A, Sütterlin M, Kapp M, Dietl J, Ruck P, Kämmerer U.** Antigen-presenting cells in human endometrium during the menstrual cycle compared to early pregnancy. *J Soc Gynecol Invest*. 2004;11:488–493.
37. **Plaks V, Birnberg T, Berkutzki T, Sela S, BenYashar A, Kalchenko V, Mor G, Keshet E, Dekel N, Neeman M, Jung S.** Uterine DCs are crucial for decidua formation during embryo implantation in mice. *J Clin Invest*. 2008;118:3954–3965.
38. **Cornillie FJ, Lauweryns JM, Brosens IA.** Normal human endometrium. An ultrastructural survey. *Gynecol Obstet Invest*. 1985;20:113–129.
39. **Lawn AM, Wilson EW, Finn CA.** The ultrastructure of human decidual and predecidual cells. *J Reprod Fertil*. 1971;26:85–90.
40. **Kajihara T, Tanaka K, Oguro T, Tochigi H, Prechapanich J, Uchino S, Itakura A, Sucurovic S, Murakami K, Brosens JJ, Ishihara O.** Androgens modulate the morphological characteristics of human endometrial stromal cells decidualized in vitro. *Reprod Sci*. 2014;21:372–380.
41. **Chen GTC, Getsios S, MacCalman CD.** Cadherin-11 is a hormonally regulated cellular marker of decidualization in human endometrial stromal cells. *Mol Reprod Dev*. 1999;52:158–165.
42. **MacCalman CD, Getsios S, Chen GTC.** Type 2 cadherins in the human endometrium and placenta: Their putative roles in human implantation and placentation. *Am J Reprod Immunol*. 1998;39:96–107.
43. **Jahn E, Classen-Linke I, Kusche M, Beier HM, Traub O, Grümmer R, Winterhager E.** Expression of gap junction connexins in the human endometrium throughout the menstrual cycle. *Hum Reprod*. 1995;10:2666–2670.
44. **Yu J, Wu J, Bagchi IC, Bagchi MK, Sidell N, Taylor RN.** Disruption of gap junctions reduces biomarkers of decidualization and angiogenesis and increases inflammatory mediators in human endometrial stromal cell cultures. *Mol Cell Endocrinol*. 2011;344:25–34.
45. **Laws MJ, Taylor RN, Sidell N, DeMayo FJ, Lydon JP, Gutstein DE, Bagchi MK, Bagchi IC.** Gap junction communication between uterine stromal cells plays a critical role in pregnancy-associated neovascularization and embryo survival. *Development*. 2008;135:2659–2668.
46. **Nair RR, Jain M, Singh K.** Reduced expression of gap junction gene connexin 43 in recurrent early pregnancy loss patients. *Placenta*. 2011;32:619–621.
47. **Iwahashi M, Muragaki Y, Ooshima A, Yamoto M, Nakano R.** Alterations in distribution and composition of the extracellular matrix during decidualization of the human endometrium. *J Reprod Fertil*. 1996;108:147–155.
48. **Wewer UM, Faber M, Liotta LA, Albrechtsen R.** Immunochemical and ultrastructural assessment of the nature of the pericellular basement membrane of human decidual cells. *Lab Invest*. 1985;53:624–633.
49. **Khong TY, Lane EB, Robertson WB.** An immunocytochemical study of fetal cells at the maternal-placental interface using monoclonal antibodies to keratins, vimentin and desmin. *Cell Tissue Res*. 1986;246:189–195.
50. **Ben-Jonathan N, Mershon JL, Allen DL, Steinmetz RW.**

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

Extrapituitary prolactin: Distribution, regulation, functions, and clinical aspects. *Endocr Rev*. 1996;17:639–669.
51. **Kletzky OA, Rossman F, Bertolli SI, Platt LD, Mishell DR, Jr.** Dynamics of human chorionic gonadotropin, prolactin, and growth hormone in serum and amniotic fluid throughout normal human pregnancy. *Am J Obstet Gynecol* 1985; 151:878–884.
52. **Golander A, Hurley T, Barrett J, Hizi A, Handwerger S.** Prolactin synthesis by human chorion-decidual tissue: A possible source of prolactin in the amniotic fluid. *Science*. 1978;202:311–313.
53. **Riddick DH, Luciano AA, Kusmik WJ, Maslar IA.** De novo synthesis of prolactin by human decidua. *Life Sci*. 1978; 23:1913–1922.
54. **Maslar IA, Riddick DH.** Prolactin production by human endometrium during the normal menstrual cycle. *Am J Obstet Gynecol*. 1979;135:751–754.
55. **Daly DC, Maslar IA, Riddick DH.** Prolactin production during in vitro decidualization of proliferative endometrium. *Am J Obstet Gynecol*. 1983;145:672–678.
56. **Braverman MB, Bagni A, de Ziegler D, Den T, Gurpide E.** Isolation of prolactin-producing cells from first and second trimester decidua. *J Clin Endocrinol Metab*. 1984;58:521–525.
57. **Wu W-X, Brooks J, Glasier AF, McNeilly AS.** The relationship between decidualization and prolactin mRNA and production at different stages of human pregnancy. *J Mol Endocrinol*. 1995;14:255–261.
58. **Clements J, Whitfeld P, Cooke N, Healy D, Matheson B, Shine J, Funder J.** Expression of the prolactin gene in human decidua-chorion. *Endocrinology*. 1983;112:1133–1134.
59. **Taii S, Ihara Y, Mori T.** Identification of the mRNA coding for prolactin in the human decidua. *Biochem Biophys Res Commun*. 1984;124:530–537.
60. **Jabbour HN, Critchley HOD.** Potential roles of decidual prolactin in early pregnancy. *Reproduction*. 2001;121: 197–205.
61. **Jabbour HN, Critchley HOD, YuLee LY, Boddy SC.** Localization of interferon regulatory factor-1 (IRF-1) in nonpregnant human endometrium: Expression of IRF-1 is upregulated by prolactin during the secretory phase of the menstrual cycle. *J Clin Endocrinol Metab*. 1999;84:4260–4265.
62. **Yu-Lee LY.** Molecular actions of prolactin in the immune system. *Proc Soc Exp Biol Med*. 1997;215:35–52.
63. **Maaskant RA, Bogic LV, Gilger S, Kelly PA, Bryant-Greenwood GD.** The human prolactin receptor in the fetal membranes, decidua, and placenta. *J Clin Endocrinol Metab*. 1996;81:396–405.
64. **Stefanoska I, Jovanovic Krivokuca M, Vasilijic S, Cujic D, Vicovac L.** Prolactin stimulates cell migration and invasion by human trophoblast *in vitro*. *Placenta*. 2013;34:775–783.
65. **Ben-Jonathan N, LaPensee CR, LaPensee EW.** What can we learn from rodents about prolactin in humans? *Endocr Rev*. 2008;29:1–41.
66. **Corbacho AM, de la Escalera GM, Clapp C.** Roles of prolactin and related members of the prolactin/growth hormone/placental lactogen family in angiogenesis. *J Endocrinol*. 2002;173:219–238.
67. **Tyson JE, Mowat GS, McCoshen JA.** Simulation of a probable biologic action of decidual prolactin on fetal membranes. *Am J Obstet Gynecol*. 1984;148:296–300.
68. **Bao L, Tessier C, Prigent-Tessier A, Li F, Buzzio OL, Callegari EA, Horseman ND, Gibori G.** Decidual prolactin silences the expression of genes detrimental to pregnancy. *Endocrinology*. 2007;148:2326–2334.
69. **Reese J, Binart N, Brown N, Ma W-G, Paria BC, Das SK, Kelly PA, Key SK.** Implantation and decidualization defects in prolactin receptor (PRLR)-deficient mice are mediated by ovarian but not uterine PRLR. *Endocrinology*. 2000;141:1872–1881.
70. **Stocco C, Telleria C, Gibori G.** The molecular control of corpus luteum formation, function, and regression. *Endocr Rev*. 2007;28:117–149.
71. **Rutanen EM, Koistinen R, Wahlstrom T, Bohn H, Ranta T, Seppala M.** Synthesis of placental protein 12 by human decidua. *Endocrinology*. 1985;116:1304–1309.
72. **Wathen NC, Campbell DJ, Patel B, Touzel R, Chard T.** Dynamics of prolactin in amniotic fluid and extraembryonic coelomic fluid in early human pregnancy. *Early Hum Dev*. 1993;35:167–172.
73. **Rutanen EM, Koistinen R, Sjöberg J, Julkunen M, Wahlströhm T, Bohn H, Seppälä M.** Synthesis of placental protein 12 by human endometrium. *Endocrinology*. 1986; 118:1067–1071.
74. **Carter AM, Hills F, O'Gorman DB, Roberts CT, Sooranna SR, Watson CS, Westwood M.** The insulin-like growth factor system in mammalian pregnancy - a workshop report. Placenta 25 Suppl. *A, Trophoblast Research, Vol*. 2004;18:S53–56.
75. **Coppock HA, White A, Aplin JD, Westwood M.** Matrix metalloprotease-3 and -9 proteolyze insulin-like growth factor-binding protein-1. *Biol Reprod*. 2004;71:438–443.
76. **Gleeson LM, Chakraborty C, McKinnon T, Lala PK.** Insulin-like growth factor-binding protein 1 stimulates human trophoblast migration by signaling through $\alpha5\beta1$ integrin via mitogen-activated protein kinase pathway. *J Clin Endocrinol Metab*. 2001;86:2484–2493.
77. **Giudice LC.** Maternal-fetal conflict – lessons from a transgene. *J Clin Invest*. 2002;110:307–309.
78. **Fazleabas AT, Kim JJ, Strakova Z.** Implantation: embryonic signals and the modulation of the uterine environment - a review. *Placenta* 2004;25 Suppl A:S26–31.
79. **Kothapalli R, Buyuksal I, Wu SQ, Chegini N, Tabibzadeh S.** Detection of *ebaf*, a novel human gene of the transforming growth factor β superfamily - Association of gene expression with endometrial bleeding. *J Clin Invest*. 1997; 99:2342–2350.
80. **Tabibzadeh S, Lessey B, Satyaswaroop PG.** Temporal and site-specific expression of transforming growth factor-$\beta4$ in human endometrium. *Mol Hum Reprod*. 1998;4:595–602.
81. **Christian M, Zhang X, Schneider-Merck T, Unterman TG, Gellersen B, White JO, Brosens JJ.** Cyclic AMP-induced forkhead transcription factor, *FKHR, cooperates with CCAAT/enhancer-binding protein β in differentiating human endometrial stromal cells*. *J Biol Chem*. 2002; 277:20825–20832.
82. **Matsuoka A, Kizuka F, Lee L, Tamura I, Taniguchi K, Asada H, Taketani T, Tamura H, Sugino N.** Progesterone

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

increases manganese superoxide dismutase expression via a cAMP-dependent signaling mediated by noncanonical Wnt5a pathway in human endometrial stromal cells. *J Clin Endocrinol Metab*. 2010;95:E291–299.

83. **Macdonald LJ, Sales KJ, Grant V, Brown P, Jabbour HN, Catalano RD.** Prokineticin 1 induces Dickkopf 1 expression and regulates cell proliferation and decidualization in the human endometrium. *Mol Hum Reprod*. 2011;17: 626–636.
84. **Evans J, Catalano RD, Morgan K, Critchley HO, Millar RP, Jabbour HN.** Prokineticin 1 signaling and gene regulation in early human pregnancy. *Endocrinology*. 2008; 149:2877–2887.
85. **Zhang XH, Liang X, Liang XH, Wang TS, Qi QR, Deng WB, Sha AG, Yang ZM.** The mesenchymal-epithelial transition during in vitro decidualization. *Reprod Sci*. 2013; 20:354–360.
86. **Uchida H, Maruyama T, Nishikawa-Uchida S, Oda H, Miyazaki K, Yamasaki A, Yoshimura Y.** Studies using an in vitro model show evidence of involvement of epithelial-mesenchymal transition of human endometrial epithelial cells in human embryo implantation. *J Biol Chem*. 2012; 287:4441–4450.
87. **Lockwood CJ, Krikun G, Papp C, Aigner S, Schatz F.** Biological mechanisms underlying the clinical effects of RU 486: modulation of cultured endometrial stromal cell plasminogen activator and plasminogen activator inhibitor expression. *J Clin Endocrinol Metab*. 1995;80:1100–1105.
88. **Lockwood CJ, Nemerson Y, Guller S, Krikun G, Alvarez M, Hausknecht V, Gurpide E, Schatz F.** Progestational regulation of human endometrial stromal cell tissue factor expression during decidualization. *J Clin Endocrinol Metab*. 1993;76:231–236.
89. **Lockwood CJ, Krikun G, Papp C, Toth-Pal E, Markiewicz L, Wang EY, Kerenyi T, Zhou X, Hausknecht V, Papp Z, et a.** The role of progestationally regulated stromal cell tissue factor and type-1 plasminogen activator inhibitor (PAI-1) in endometrial hemostasis and menstruation. *Ann N Y Acad Sci* 1994;734:57–79.
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.
91. **Lockwood CJ, Krikun G, Schatz F.** Human endometrial hemostasis during the menstrual cycle and gestation. In: Aplin JD, Fazelabas AT, Glasser SR, Giudice LC eds. The Endometrium: Molecular, Cellular, Clinical Perspectives. 2nd ed. London: Informa Healthcare; 2008;397–408.
92. **Pohnke Y, Schneider-Merck T, Fahnenstich J, Kempf R, Christian M, Milde-Langosch K, Brosens JJ, Gellersen B.** Wild-type p53 protein is up-regulated upon cyclic AMP-induced differentiation of human endometrial stromal cells. *J Clin Endocrinol Metab*. 2004;89:5233–5244.
93. **Gellersen B, Briese J, Oberndörfer M, Redlin K, Samalecos A, Richter D-U, Löning T, Schulte HM, Bamberger AM.** Expression of the metastasis suppressor KAI-1 in decidual cells at the human maternal-fetal interface: Regulation and functional implications. *Am J Pathol*. 2007;170:126–139.
94. **Fazleabas AT, Strakova Z.** Endometrial function: cell specific changes in the uterine environment. *Mol Cell Endocrinol*. 2002;186:143–147.
95. **Schwenke M, Knöfler M, Velicky P, Weimar CHE, Kruse M, Samalecos A, Wolf A, Macklon NS, Bamberger AM, Gellersen B.** Control of human endometrial stromal cell motility by PDGF-BB, *HB-EGF and trophoblast-secreted factors. PLoS ONE*. 2013;8:e54336.
96. **Ihnatovych I, Hu W, Martin JL, Fazleabas AT, de Lanerolle P, Strakova Z.** Increased phosphorylation of myosin light chain prevents *in vitro* decidualization. *Endocrinology*. 2007;148:3176–3184.
97. **Brar A, Handwerger S, Kessler CA, Aronow BJ.** Gene induction and categorical reprogramming during in vitro human endometrial fibroblast decidualization. *Physiol Genomics*. 2001;7:135–148.
98. **Tierney EP, Tulac S, Huang ST, Giudice LC.** Activation of the protein kinase A pathway in human endometrial stromal cells reveals sequential categorical gene regulation. *Physiol Genomics*. 2003;16:47–66.
99. **Giudice LC.** Microarray expression profiling reveals candidate genes for human uterine receptivity. *Am J Pharmacogenomics*. 2004;4:299–312.
100. **Lu Z, Hardt J, Kim JJ.** Global analysis of genes regulated by HOXA10 in decidualization reveals a role in cell proliferation. *Mol Hum Reprod*. 2008;14:357–366.
101. **Aghajanova L, Horcajadas JA, Weeks JL, Esteban FJ, Nezhat CN, Conti M, Giudice LC.** The protein kinase A pathway-regulated transcriptome of endometrial stromal fibroblasts reveals compromised differentiation and persistent proliferative potential in endometriosis. *Endocrinology*. 2010;151:1341–1355.
102. **Takano M, Lu Z, Goto T, Fusi L, Higham J, Francis J, Whitey A, Hardt J, Cloke B, Stavropoulou AV, Ishihara O, Lam EW-F, Unterman TG, Brosens JJ, Kim JJ.** Transcriptional cross talk between the forkhead transcription factor box O1A and the progesterone receptor coordinates cell cycle regulation and differentiation in human endometrial stromal cells. *Mol Endocrinol*. 2007;21:2334–2349.
103. **Cloke B, Huhtinen K, Fusi L, Kajihara T, Yliheikkila M, Ho KK, Teklenburg G, Lavery S, Jones MC, Trew G, Kim JJ, Lam EW, Cartwright JE, Poutanen M, Brosens JJ.** The androgen and progesterone receptors regulate distinct gene networks and cellular functions in decidualizing endometrium. *Endocrinology*. 2008;149:4462–4474.
104. **Leitao B, Jones MC, Fusi L, Higham J, Lee Y, Takano M, Goto T, Christian M, Lam EW, Brosens JJ.** Silencing of the JNK pathway maintains progesterone receptor activity in decidualizing human endometrial stromal cells exposed to oxidative stress signals. *FASEB J*. 2010;24:1541–1551.
105. **Grimaldi G, Christian M, Steel JH, Henriet P, Poutanen M, Brosens JJ.** Down-regulation of the histone methyltransferase EZH2 contributes to the epigenetic programming of decidualizing human endometrial stromal cells. *Mol Endocrinol*. 2011;25:1892–1903.
106. **Paule SG, Airey LM, Li Y, Stephens AN, Nie G.** Proteomic approach identifies alterations in cytoskeletal remodelling proteins during decidualization of human endometrial stromal cells. *J Proteome Res*. 2010;9:5739–5747.
107. **Gellersen B, Wolf A, Kruse M, Schwenke M, Bamberger AM.** Human endometrial stromal cell – trophoblast interactions: Mutual stimulation of chemotactic migration and pro-migratory roles of cell surface molecules CD82 and CEACAM1. *Biol Reprod*. 2013;88:80.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

108. **Garrido-Gomez T, Dominguez F, Lopez JA, Camafeita E, Quinonero A, Martinez-Conejero JA, Pellicer A, Conesa A, Simon C.** Modeling human endometrial decidualization from the interaction between proteome and secretome. *J Clin Endocrinol Metab*. 2011;96:706–716.
109. **Prianishnikov VA.** On the concept of stem cell and a model of functional-morphological structure of the endometrium. *Contraception*. 1978;18:213–223.
110. **Chan RWS, Schwab KE, Gargett CE.** Clonogenicity of human endometrial epithelial and stromal cells. *Biol Reprod*. 2004;70:1738–1750.
111. **Maruyama T, Yoshimura Y.** Stem cell theory for the pathogenesis of endometriosis. *Front Biosci (Elite Ed)*. 2012;4:2854–2863.
112. **Gargett CE, Schwab KE, Zillwood RM, Nguyen HP, Wu D.** Isolation and culture of epithelial progenitors and mesenchymal stem cells from human endometrium. *Biol Reprod*. 2009;80:1136–1145.
113. **Gargett CE, Ye L.** Endometrial reconstruction from stem cells. *Fertil Steril*. 2012;98:11–20.
114. **Kanematsu D, Shofuda T, Yamamoto A, Ban C, Ueda T, Yamasaki M, Kanemura Y.** Isolation and cellular properties of mesenchymal cells derived from the decidua of human term placenta. *Differentiation*. 2011;82:77–88.
115. **Macias MI, Grande J, Moreno A, Dominguez I, Bornstein R, Flores AI.** Isolation and characterization of true mesenchymal stem cells derived from human term decidua capable of multilineage differentiation into all 3 embryonic layers. *Am J Obstet Gynecol* 2010;203:495 e499–495 e423.
116. **Cervello I, Gil-Sanchis C, Mas A, Faus A, Sanz J, Moscardo F, Higueras G, Sanz MA, Pellicer A, Simon C.** Bone marrow-derived cells from male donors do not contribute to the endometrial side population of the recipient. *PLoS ONE*. 2012;7:e30260.
117. **Morelli SS, Rameshwar P, Goldsmith LT.** Experimental evidence for bone marrow as a source of nonhematopoietic endometrial stromal and epithelial compartment cells in a murine model. *Biol Reprod*. 2013;89:7.
118. **Masuda H, Anwar SS, Buhring HJ, Rao JR, Gargett CE.** A novel marker of human endometrial mesenchymal stem-like cells. *Cell Transplant*. 2012;21:2201–2214.
119. **Muñoz-Fernández R, Prados A, Leno-Duran E, Blazquez A, Garcia-Fernandez JR, Ortiz-Ferron G, Olivares EG.** Human decidual stromal cells secrete C-X-C motif chemokine 13, express B cell-activating factor and rescue B lymphocytes from apoptosis: distinctive characteristics of follicular dendritic cells. *Hum Reprod*. 2012;27:2775–2784.
120. **Krautler NJ, Kana V, Kranich J, Tian Y, Perera D, Lemm D, Schwarz P, Armulik A, Browning JL, Tallquist M, Buch T, Oliveira-Martins JB, Zhu C, Hermann M, Wagner U, Brink R, Heikenwalder M, Aguzzi A.** Follicular dendritic cells emerge from ubiquitous perivascular precursors. *Cell*. 2012;150:194–206.
121. **Challen GA, Little MH.** A side order of stem cells: the SP phenotype. *Stem Cells*. 2006;24:3–12.
122. **Tsuji S, Yoshimoto M, Takahashi K, Noda Y, Nakahata T, Heike T.** Side population cells contribute to the genesis of human endometrium. *Fertil Steril*. 2008;90:1528–1537.
123. **Cervello I, Mas A, Gil-Sanchis C, Peris L, Faus A, Saunders PT, Critchley HO, Simon C.** Reconstruction of endometrium from human endometrial side population cell lines. *PLoS ONE*. 2011;6:e21221.
124. **Sivasubramaniyan K, Harichandan A, Schumann S, Sobiesiak M, Lengerke C, Maurer A, Kalbacher H, Buhring HJ.** Prospective isolation of mesenchymal stem cells from human bone marrow using novel antibodies directed against Sushi domain containing 2. *Stem Cells Dev*. 2013;22:1944–1954.
125. **Valentijn AJ, Palial K, Al-Lamee H, Tempest N, Drury J, Von Zglinicki T, Saretzki G, Murray P, Gargett CE, Hapangama DK.** SSEA-1 isolates human endometrial basal glandular epithelial cells: phenotypic and functional characterization and implications in the pathogenesis of endometriosis. *Hum Reprod*. 2013;28:2695–2708.
126. **Huang CC, Orvis GD, Wang Y, Behringer RR.** Stromal-to-epithelial transition during postpartum endometrial regeneration. *PLoS ONE*. 2012;7:e44285.
127. **Patterson AL, Zhang L, Arango NA, Teixeira J, Pru JK.** Mesenchymal-to-epithelial transition contributes to endometrial regeneration following natural and artificial decidualization. *Stem Cells Dev*. 2013;22:964–974.
128. **Musina RA, Belyavski AV, Tarusova OV, Solovyova EV, Sukhikh GT.** Endometrial mesenchymal stem cells isolated from the menstrual blood. *Bull Exp Biol Med*. 2008;145:539–543.
129. **Meng X, Ichim TE, Zhong J, Rogers A, Yin Z, Jackson J, Wang H, Ge W, Bogin V, Chan KW, Thebaud B, Riordan NH.** Endometrial regenerative cells: a novel stem cell population. *J Transl Med*. 2007;5:57.
130. **Patel AN, Park E, Kuzman M, Benetti F, Silva FJ, Allickson JG.** Multipotent menstrual blood stromal stem cells: isolation, characterization, and differentiation. *Cell Transplant*. 2008;17:303–311.
131. **Bockeria L, Bogin V, Bockeria O, Le T, Alekyan B, Woods EJ, Brown AA, Ichim TE, Patel AN.** Endometrial regenerative cells for treatment of heart failure: a new stem cell enters the clinic. *J Transl Med*. 2013;11:56.
132. **Vegh I, Grau M, Gracia M, Grande J, de la Torre P, Flores AI.** Decidua mesenchymal stem cells migrated toward mammary tumors *in vitro* and i*n vivo* affecting tumor growth and tumor development. *Cancer Gene Ther*. 2013;20:8–16.
133. **Murakami K, Bhandari H, Lucas ES, Takeda S, Gargett CE, Quenby S, Brosens JJ, Tan BK.** Deficiency in clonogenic endometrial mesenchymal stem cells in obese women with reproductive failure - a pilot study. *PLoS ONE*. 2013;8:e82582.
134. **Johansson ED, Wide L.** Periovulatory levels of plasma progesterone and luteinizing hormone in women. *Acta Endocrinol (Copenh)*. 1969;62:82–88.
135. **Dawood MY, Saxena BB.** Plasma testosterone and dihydrotestosterone in ovulatory and anovulatory cycles. *Am J Obstet Gynecol*. 1976;126:430–435.
136. **Vermeulen A, Verdonck L.** Plasma androgen levels during the menstrual cycle. *Am J Obstet Gynecol*. 1976;125:491–494.
137. **Salonia A, Pontillo M, Nappi RE, Zanni G, Fabbri F, Scavini M, Daverio R, Gallina A, Rigatti P, Bosi E, Bonini PA, Montorsi F.** Menstrual cycle-related changes in circu-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

lating androgens in healthy women with self-reported normal sexual function. *J Sex Med*. 2008;5:854–863.
138. **Huhtinen K, Desai R, Stahle M, Salminen A, Handelsman DJ, Perheentupa A, Poutanen M.** Endometrial and endometriotic concentrations of estrone and estradiol are determined by local metabolism rather than circulating levels. *J Clin Endocrinol Metab*. 2012;97:4228–4235.
139. **Guerrero R, Landgren BM, Montiel R, Cekan Z, Diczfalusy E.** Unconjugated steroids in the human endometrium. *Contraception*. 1975;11:169–177.
140. **Gellersen B, Brosens JJ.** Cyclic AMP and progesterone receptor cross-talk in human endometrium: a decidualizing affair. *J Endocrinol*. 2003;178:357–372.
141. **Maslar IA, Ansbacher R.** Effects of progesterone on decidual prolactin production by organ cultures of human endometrium. *Endocrinology*. 1986;118:2102–2108.
142. **Han SW, Lei ZM, Rao CV.** Treatment of human endometrial stromal cells with chorionic gonadotropin promotes their morphological and functional differentiation into decidua. *Mol Cell Endocrinol*. 1999;147:7–16.
143. **Tang B, Gurpide E.** Direct effect of gonadotropins on decidualization of human endometrial stromal cells. *J Steroid Biochem Mol Biol*. 1993;47:115–121.
144. **Kasahara K, Takakura K, Takebayashi K, Kimura F, Nakanishi K, Noda Y.** The role of human chorionic gonadotropin on decidualization of endometrial stromal cells *in vitro*. *J Clin Endocrinol Metab*. 2001;86:1281–1286.
145. **Kajihara T, Tochigi H, Uchino S, Itakura A, Brosens JJ, Ishihara O.** Differential effects of urinary and recombinant chorionic gonadotropin on oxidative stress responses in decidualizing human endometrial stromal cells. *Placenta*. 2011;32:592–597.
146. **Tabanelli S, Tang B, Gurpide E.** *In vitro decidualization of human endometrial stromal cells. J Steroid Biochem Mol Biol*. 1992;42:337–344.
147. **Lane B, Oxberry W, Mazella J, Tseng L.** Decidualization of human endometrial stromal cells in vitro: effects of progestin and relaxin on the ultrastructure and production of decidual secretory proteins. *Hum Reprod*. 1994;9:259–266.
148. **Stewart DR, Celniker AC, Taylor Jr CA, Cragun JR, Overstreet JW, Lasley BL.** Relaxin in the peri-implantation period. *J Clin Endocrinol Metab*. 1990;70:1771–1773.
149. **Johnson MR, Carter G, Grint C, Lightman SL.** Relationship between ovarian steroids, gonadotrophins and relaxin during the menstrual cycle. *Acta Endocrinol (Copenh)*. 1993;129:121–125.
150. **Palejawa S, Tseng L, Wojtczuk A, Weiss G, Goldsmith LT.** Relaxin gene and protein expression and its regulation of procollagenase and vascular endothelial growth factor in human endometrial cells. *Biol Reprod*. 2002;66:1743–1748.
151. **Ivell R, Balvers M, Pohnke Y, Telgmann R, Bartsch O, Milde-Langosch K, Bamberger AM, Einspanier.** A Immunoexpression of the relaxin receptor LGR7 in breast and uterine tissues of humans and primates. *Reprod Biol Endocrinol*. 2003;1:114.
152. **Gravanis A, Stournaras C, Margioris AN.** Paracrinology of endometrial neuropeptides: corticotropin-releasing hormone and opioids. *Semin Reprod Endocrinol*. 1999;17:29–38.
153. **Milne SA, Perchick GB, Boddy SC, Jabbour HN.** Expression, localization, and signaling of PGE_2 and EP2/EP4 receptors in human nonpregnant endometrium across the menstrual cycle. *J Clin Endocrinol Metab*. 2001;86:4453–4459.
154. **Tabibzadeh S, Sun XZ, Kong QF, Kasnic G, Miller J, Satyaswaroop PG.** Induction of a polarized micro-environment by human T cells and interferon-gamma in three-dimensional spheroid cultures of human endometrial epithelial cells. *Hum Reprod*. 1993;8:182–192.
155. **Stewart CJ, Farquharson MA, Foulis AK.** The distribution and possible function of gamma interferon-immunoreactive cells in normal endometrium and myometrium. *Virchows Arch A Pathol Anat Histopathol*. 1992;420:419–424.
156. **Christian M, Marangos P, Mak I, McVey J, Barker F, White J, Brosens JJ.** Interferon-γ modulates prolactin and tissue factor expression in differentiating human endometrial stromal cells. *Endocrinology*. 2001;142:3142–3151.
157. **Lejeune B, Van Hoeck J, Leroy F.** Transmitter role of the luminal uterine epithelium in the induction of decidualization in rats. *J Reprod Fertil*. 1981;61:235–240.
158. **Dimitriadis E, Salamonsen LA, Robb L.** Expression of interleukin-11 during the human menstrual cycle: coincidence with stromal cell decidualization and relationship to leukaemia inhibitory factor and prolactin. *Mol Hum Reprod*. 2000;6:907–914.
159. **Dimitriadis E, Stoikos C, Baca M, Fairlie WD, McCoubrie JE, Salamonsen LA.** Relaxin and prostaglandin E_2 regulate interleukin 11 during human endometrial stromal cell decidualization. *J Clin Endocrinol Metab*. 2005;90:3458–3465.
160. **Karpovich N, Klemmt P, Hwang JH, McVeigh JE, Heath JK, Barlow DH, Mardon HJ.** The production of interleukin-11 and decidualization are compromised in endometrial stromal cells derived from patients with infertility. *J Clin Endocrinol Metab*. 2005;90:1607–1612.
161. **Robb L, Li R, Hartley L, Nandurkar HH, Koentgen R, Begley CG.** Infertility in female mice lacking the receptor for interleukin 11 is due to a defective uterine response to implantation. *Nat Med*. 1998;4:303–3308.
162. **Strakova Z, Mavrogianis P, Meng X, Hastings JM, Jackson KS, Cameo P, Brudney A, Knight O, Fazleabas AT.** In vivo infusion of interleukin-1β and chorionic gonadotropin induces endometrial changes that mimic early pregnancy events in the baboon. *Endocrinology*. 2005;146:4097–4104.
163. **Geisert R, Fazleabas A, Lucy M, Mathew D.** Interaction of the conceptus and endometrium to establish pregnancy in mammals: role of interleukin 1β. *Cell Tissue Res*. 2012;349:825–838.
164. **van Mourik MS, Macklon NS, Heijnen CJ.** Embryonic implantation: cytokines, adhesion molecules, and immune cells in establishing an implantation environment. *J Leukoc Biol*. 2009;85:4–19.
165. **Kimber SJ.** Blastocyst implantation: the adhesion cascade. In: Aplin JD, Fazelabas AT, Glasser SR, Giudice LC eds. The Endometrium: Molecular, Cellular, Clinical Perspectives. 2nd ed. London: Informa Healthcare; 2008;331–351.
166. **Hwang JH, Park MI, Hwang YY, Yoo HJ, Mardon HJ.**

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

The characteristics of integrins expression in decidualized human endometrial stromal cell induced by 8-Br-cAMP in *in vitro*. *Exp Mol Med*. 2002;34:194–200.

167. **Gonzalez M, Neufeld J, Reimann K, Wittmann S, Samalecos A, Wolf A, Bamberger AM, Gellersen B.** Expansion of human trophoblastic spheroids is promoted by decidualized endometrial stromal cells and enhanced by heparin-binding epidermal growth factor-like growth factor and interleukin-1® Mol Hum Reprod. 2011;17:421–433.
168. **Tamura M, Sebastian S, Yang S, Gurates B, Fang Z, Bulun SE.** Interleukin-1β elevates cycloocygenase-2 protein level and enzyme activity via increasing its mRNA stability in human endometrial stromal cells: an effect mediated by extracellularly regulated kinases 1 and 2. *J Clin Endocrinol Metab*. 2002;87:3263–3273.
169. **Krüssel JS, Bielfeld P, Polan ML, Simon C.** Regulation of embryonic implantation. *Eur J Obstet Gynecol Reprod Biol 110 Suppl*. 2003;1:S2–9.
170. **Hess AP, Hamilton AE, Talbi S, Dosiou C, Nyegaard M, Nayak N, Genbacev-Krtolica O, Mavrogianis P, Ferrer K, Kruessel J, Fazleabas AT, Fisher SJ, Giudice LC.** Decidual stromal cell response to paracrine signals from the trophoblast: Amplification of immune and angiogenic modulators. *Biol Reprod*. 2007;76:102–117.
171. **Frank GR, Brar AK, Jikihara H, Cedars MI, Handwerger S.** Interleukin-1β and the endometrium: an inhibitor of stromal cell differentiation and possible autoregulator of decidualization in humans. *Biol Reprod*. 1995;52:184–191.
172. **Strakova Z, Srisuparp S, Fazleabas AT.** Interleukin-1β induces the expression of insulin-like growth factor binding protein-1 during decidualization in the primate. *Endocrinology*. 2000;141:4664–4670.
173. **Baston-Büst DM, Götte M, Janni W, Krüssel JS, Hess AP.** Syndecan-1 knock-down in decidualized human endometrial stromal cells leads to significant changes in cytokine and angiogenic factor expression patterns. *Reprod Biol Endocrinol*. 2010;8:133.
174. **Nasu K, Fujisawa K, Arima K, Kai K, Sugano T, Miyakawa I.** Expression and regulation of growth-regulated oncogene α in human endometrial stromal cells. *Mol Hum Reprod*. 2001;7:741–746.
175. **Baston-Büst DM, Schanz A, Boddeker SJ, Altergot-Ahmad O, Krüssel JS, Rein D, Hess AP.** CXCL1 expression in human decidua in vitro is mediated via the MAPK signalling cascade. *Cytokine*. 2013;64:79–85.
176. **Mulayim N, Palter SF, Kayisli UA, Senturk L, Arici A.** Chemokine receptor expression in human endometrium. *Biol Reprod*. 2003;68:1491–1495.
177. **Scapini P, Morini M, Tecchio C, Minghelli S, Di Carlo E, Tanghetti E, Albini A, Lowell C, Berton G, Noonan DM, Cassatella MA.** CXCL1/macrophage inflammatory protein-2-induced angiogenesis in vivo is mediated by neutrophil-derived vascular endothelial growth factor-A. *J Immunol*. 2004;172:5034–5040.
178. **Germeyer A, Sharkey AM, Prasadajudio M, Sherwin R, Moffett A, Bieback K, Clausmeyer S, Masters L, Popovici RM, Hess AP, Strowitzki T, von Wolff M.** Paracrine effects of uterine leucocytes on gene expression of human uterine stromal fibroblasts. *Mol Hum Reprod*. 2009;15:39–48.
179. **Popovici RN, Kao L-C, Giudice LC.** Discovery of new inducible genes in *in vitro* decidualized human endometrial stromal cells using microarray technology. *Endocrinology*. 2000;141:3510–3513.
180. **Popovici RM, Betzler NK, Krause MS, Luo M, Jauckus J, Germeyer A, Bloethner S, Schlotterer A, Kumar R, Strowitzki T, von Wolff M.** Gene expression profiling of human endometrial-trophoblast interaction in a coculture model. *Endocrinology*. 2006;147:5662–5675.
181. **Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi I, Köntgen F, Abbondanzo SJ.** Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature*. 1992;359:76–79.
182. **Dey SK, Lim H, K DS, Reese J, Paria BC, Daikoku T, Wang H.** Molecular cues to implantation. *Endocr Rev*. 2004;25:341–373.
183. **Cullinan EB, Abbondanzo SJ, Anderson PS, Pollard JW, Lessey BA, Stewart CL.** Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine paracrine function in regulating embryo implantation. *Proc Nat Acad Sci USA*. 1996;93:3115–3120.
184. **Shuya LL, Menkhorst EM, Yap J, Li P, Lane N, Dimitriadis E.** Leukemia inhibitory factor enhances endometrial stromal cell decidualization in humans and mice. *PLoS ONE*. 2011;6:e25288.
185. **Brinsden PR, Alam V, de Moustier B, Engrand P.** Recombinant human leukemia inhibitory factor does not improve implantation and pregnancy outcomes after assisted reproductive techniques in women with recurrent unexplained implantation failure. *Fertil Steril*. 2009;91:1445–1447.
186. **Chobotova K, Spyropoulou I, Carver J, Manek S, Heath JK, Gullick WJ, Barlow DH, Sargent IL, Mardon HJ.** Heparin-binding epidermal growth factor and its receptor ErbB4 mediate implantation of the human blastocyst. *Mech Dev*. 2002;119:137–144.
187. **Lessey BA, Gui Y, Apparao KB, Young SL, Mulholland J.** Regulated expression of heparin-binding EGF-like growth factor (HB-EGF) in the human endometrium: a potential paracrine role during implantation. *Mol Reprod Dev*. 2002;62:446–455.
188. **Chobotova K, Karpovich N, Carver J, Manek S, Gullick WJ, Barlow DH, Mardon HJ.** Heparin-binding epidermal growth factor and its receptors mediate decidualization and potentiate survival of human endometrial stromal cells. *J Clin Endocrinol Metab*. 2005;90:913–919.
189. **Xie H, Wang H, Tranguch S, Iwamoto R, Mekada E, Demayo FJ, Lydon JP, Das SK, Dey SK.** Maternal heparin-binding-EGF deficiency limits pregnancy success in mice. *Proc Natl Acad Sci USA*. 2007;104:18315–18320.
190. **Lim HJ, Dey SK.** HB-EGF: a unique mediator of embryo-uterine interactions during implantation. *Exp Cell Res*. 2009;315:619–626.
191. **Paria BC, Ma W, Tan J, Raja S, Das SK, Dey SK, Hogan BL.** Cellular and molecular responses of the uterus to embryo implantation can be elicited by locally applied growth factors. *Proc Natl Acad Sci USA*. 2001;98:1047–1052.
192. **Jones RL, Salamonsen LA, Findlay JK.** Potential roles for endometrial inhibins, activins and follistatin during human embryo implantation and early pregnancy. *Trends Endocrinol Metab*. 2002;13:144–150.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

193. **Jones RL, Stoikos C, Findlay JK, Salamonsen LA.** TGF-β superfamily expression and actions in the endometrium and placenta. *Reproduction*. 2006;132:217–232.
194. **Jones RL, Salamonsen LA, Findlay JK.** Activin A promotes human endometrial stromal cell decidualization *in vitro*. *J Clin Endocrinol Metab*. 2002;87:4001–4004.
195. **Jones RL, Salamonsen LA, Zhao YC, Ethier JF, Drummond AE, Findlay JK.** Expression of activin receptors, follistatin and betaglycan by human endometrial stromal cells; consistent with a role for activins during decidualization. *Mol Hum Reprod*. 2002;8:363–374.
196. **Jones RL, Findlay JK, Farnworth PG, Robertson DM, Wallace E, Salamonsen LA.** Activin A and inhibin A differentially regulate human uterine matrix metalloproteinases: potential interactions during decidualization and trophoblast invasion. *Endocrinology*. 2006;147:724–732.
197. **Kim MR, Park DW, Lee JH, Choi DS, Hwang KJ, Ryu HS, Min CK.** Progesterone-dependent release of transforming growth factor-beta1 from epithelial cells enhances the endometrial decidualization by turning on the Smad signalling in stromal cells. *Mol Hum Reprod*. 2005;11:801–808.
198. **Arici A, MacDonald PC, Casey ML.** Modulation of the levels of transforming growth factor β messenger ribonucleic acids in human endometrial stromal cells. *Biol Reprod*. 1996;54:463–469.
199. **Li Q, Kannan A, Wang W, DeMayo FJ, Taylor RN, Bagchi MK, Bagchi IC.** Bone morphogenetic protein 2 functions via a conserved signaling pathway involving Wnt4 to regulate uterine decidualization in the mouse and the human. *J Biol Chem*. 2007;282:31725–31732.
200. **Lee KY, Jeong JW, Wang J, Ma L, Martin JF, Tsai SY, Lydon JP, DeMayo FJ.** Bmp2 is critical for the murine uterine decidual response. *Mol Cell Biol*. 2007;27:5468–5478.
201. **Franco HL, Dai D, Lee KY, Rubel CA, Roop D, Boerboom D, Jeong JW, Lydon JP, Bagchi IC, Bagchi MK, DeMayo FJ.** WNT4 is a key regulator of normal postnatal uterine development and progesterone signaling during embryo implantation and decidualization in the mouse. *FASEB J*. 2011;25:1176–1187.
202. **Clementi C, Tripurani SK, Large MJ, Edson MA, Creighton CJ, Hawkins SM, Kovanci E, Kaartinen V, Lydon JP, Pangas SA, DeMayo FJ, Matzuk MM.** Activin-like kinase 2 functions in peri-implantation uterine signaling in mice and humans. *PLoS Genet* 2013;9:e1003863.
203. **Li Q, Kannan A, Das A, Demayo FJ, Hornsby PJ, Young SL, Taylor RN, Bagchi MK, Bagchi IC.** WNT4 acts downstream of BMP2 and functions via β-catenin signaling pathway to regulate human endometrial stromal cell differentiation. *Endocrinology*. 2013;154:446–457.
204. **Park CB, Dufort D.** NODAL signaling components regulate essential events in the establishment of pregnancy. *Reproduction*. 2013;145:R55–64.
205. **Okada H, Nie G, Salamonsen LA.** Requirement for proprotein convertase 5/6 during decidualization of human endometrial stromal cells *in vitro*. *J Clin Endocrinol Metab*. 2005;90:1028–1034.
206. **Tang M, Mikhailik A, Pauli I, Giudice LC, Fazelabas AT, Tulac S, Carson DD, Kaufman DG, Barbier C, Creemers JW, Tabibzadeh S.** Decidual differentiation of stromal cells promotes *PC5/6* expression and lefty processing. *Endocrinology*. 2005;146:5313–5320.
207. **Cornet PB, Picquet C, Lemoine P, Osteen KG, Bruner-Tran KL, Tabibzadeh S, Courtoy PJ, Eeckhout Y, Marbaix E, Henriet P.** Regulation and function of LEFTY-A/EBAF in the human endometrium. mRNA expression during the menstrual cycle, control by progesterone, and effect on matrix metalloprotineases. *J Biol Chem*. 2002;277:42496–42504.
208. **Papageorgiou I, Nicholls PK, Wang F, Lackmann M, Makanji Y, Salamonsen LA, Robertson DM, Harrison CA.** Expression of nodal signalling components in cycling human endometrium and in endometrial cancer. *Reprod Biol Endocrinol*. 2009;7:122.
209. **Park CB, DeMayo FJ, Lydon JP, Dufort D.** NODAL in the uterus is necessary for proper placental development and maintenance of pregnancy. *Biol Reprod*. 2012;86:194.
210. **Tang M, Taylor HS, Tabibzadeh S.** In vivo gene transfer of lefty leads to implantation failure in mice. *Hum Reprod*. 2005;20:1772–1778.
211. **Tang M, Naidu D, Hearing P, Handwerger S, Tabibzadeh S.** LEFTY, a member of the transforming growth factor-β superfamily, inhibits uterine stromal cell differentiation: a novel autocrine role. *Endocrinology*. 2010;151:1320–1330.
212. **Kopan R.** Notch signaling. *Cold Spring Harb Perspect Biol*. 2012;4:a011213.
213. **Afshar Y, Jeong JW, Roqueiro D, DeMayo F, Lydon J, Radtke F, Radnor R, Miele L, Fazleabas A.** Notch1 mediates uterine stromal differentiation and is critical for complete decidualization in the mouse. *FASEB J*. 2012;26:282–294.
214. **Mikhailik A, Mazella J, Liang S, Tseng L.** Notch ligand-dependent gene expression in human endometrial stromal cells. *Biochem Biophys Res Commun*. 2009;388:479–482.
215. **Afshar Y, Miele L, Fazleabas AT.** Notch1 is regulated by chorionic gonadotropin and progesterone in endometrial stromal cells and modulates decidualization in primates. *Endocrinology*. 2012;153:2884–2896.
216. **Handwerger S, Markoff E, Richards R.** Regulation of the synthesis and release of decidual prolactin by placental and autocrine/paracrine factors. *Placenta*. 1991;12:121–130.
217. **Sun X, Dey SK.** Endocannabinoid signaling in female reproduction. *ACS Chem Neurosci*. 2012;3:349–355.
218. **Kessler CA, Moghadam KK, Schroeder JK, Buckley AR, Brar AK, Handwerger S.** Cannabinoid receptor I activation markedly inhibits human decidualization. *Mol Cell Endocrinol*. 2005;229:65–74.
219. **Liu Y, Su Y, Wang X.** Phosphatidic acid-mediated signaling. *Adv Exp Med Biol*. 2013;991:159–176.
220. **Yoon MS, Koo JB, Jeong YG, Kim YS, Lee JH, Yun HJ, Lee KS, Han JS.** Phospholipase D1 as a key enzyme for decidualization in human endometrial stromal cells. *Biol Reprod*. 2007;76:250–258.
221. **Skaznik-Wikiel ME, Kaneko-Tarui T, Kashiwagi A, Pru JK.** Sphingosine-1-phosphate receptor expression and signaling correlate with uterine prostaglandin-endoperoxide synthase 2 expression and angiogenesis during early pregnancy. *Biol Reprod*. 2006;74:569–576.
222. **Mizugishi K, Li C, Olivera A, Bielawski J, Bielawska A,**

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

Deng CX, Proia RL. Maternal disturbance in activated sphingolipid metabolism causes pregnancy loss in mice. *J Clin Invest*. 2007;117:2993–3006.
223. **Liu AMF, Wong YH.** Activation of nuclear factor κB by somatostatin type 2 receptor in pancreatic acinar AR42J cells involves $G\alpha_{14}$ and multiple signaling components: a mechanism requiring protein kinase C, calmodulin-dependent kinase II, *Erk, and c-Src*. *J Biol Chem*. 2005;280:34617–34625.
224. **Tanaka K, Minoura H, Isobe T, Yonaha H, Kawato H, Wang DF, Yoshida T, Kojima M, Kangawa K, Toyoda N.** Ghrelin is involved in the decidualization of human endometrial stromal cells. *J Clin Endocrinol Metab*. 2003;88:2335–2340.
225. **Tawadros N, Salamonsen LA, Dimitriadis E, Chen C.** Facilitation of decidualization by locally produced ghrelin in the human endometrium. *Mol Hum Reprod*. 2007;13:483–489.
226. **Harada M, Osuga Y, Takemura Y, Yoshino O, Koga K, Hirota Y, Hirata T, Morimoto C, Yano T, Taketani Y.** Mechanical stretch upregulates IGFBP-1 secretion from decidualized endometrial stromal cells. *Am J Physiol Endocrinol Metab*. 2006;290:E268–272.
227. **Blithe DL, Richards RG, Skarulis MC.** Free alpha molecules from pregnancy stimulate secretion of prolactin from human decidual cells: a novel function for free alpha in pregnancy. *Endocrinology*. 1991;129:2257–2259.
228. **Thrailkill KM, Golander A, Underwood LE, Handwerger S.** Insulin-like growth factor I stimulates the synthesis and release of prolactin from human decidual cells. *Endocrinology*. 1988;123:2930–2934.
229. **Pihoker C, Feeney RJ, Su JL, Handwerger S.** Lipocortin-I inhibits the synthesis and release of prolactin from human decidual cells: Evidence for autocrine/paracrine regulation by lipocortin-I. *Endocrinology*. 1991;128:1123–1128.
230. **Eyal O, Jomain JB, Kessler C, Goffin V, Handwerger S.** Autocrine prolactin inhibits human uterine decidualization: a novel role for prolactin. *Biol Reprod*. 2007;76:777–783.
231. **Sherafat-Kazemzadeh R, Schroeder JK, Kessler CA, Handwerger S.** Parathyroid hormone-like hormone (PTHLH) represses decidualization of human uterine fibroblast cells by an autocrine/paracrine mechanism. *J Clin Endocrinol Metab*. 2011;96:509–514.
232. **Hoshi S, Morimoto T, Saito H, Ichizuka K, Matsuoka R, Yanaihara A, Suzuki M, Yanaihara T, Okai T.** PTHrP and PTH/PTHrP receptor expressions in human endometrium. *Endocr J*. 2001;48:219–225.
233. **Casey ML, Mibe M, MacDonald PC.** Regulation of parathyroid hormone-related protein gene expression in human endometrial stromal cells in culture. *J Clin Endocrinol Metab*. 1993;77:188–194.
234. **Casey ML, Erk A, MacDonald PC.** Parathyroid hormone and parathyroid hormone-related protein stimulate adenylate cyclase in human endometrial stromal cells. *Ann N Y Acad Sci*. 1994;734:365–371.
235. **Turing AM.** The chemical basis of morphogenesis. *Phil Transact Royal Soc B*. 1952;237:37–72.
236. **Brosens JJ, Tullet J, Varshochi R, Lam EW.** Steroid receptor action. *Best Pract Res Clin Obstet Gynaecol*. 2004;18:265–283.
237. **Beato M, Klug J.** Steroid hormone receptors: an update. *Hum Reprod Update*. 2000;6:225–236.
238. **Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H, Chambon P.** Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *EMBO J*. 1990;9:1603–1614.
239. **Samalecos A, Gellersen B.** Systematic expression analysis and antibody screening do not support the existence of naturally occurring progesterone receptor (PR)-C, PR-M, or other truncated PR isoforms. *Endocrinology*. 2008;149:5872–5887.
240. **Vegeto E, Shahbaz MM, Wen DX, Goldman ME, O'Malley BW, McDonnell DP.** Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. *Mol Endocrinol*. 1993;7:1244–1255.
241. **Richer JK, Jacobson BM, Manning NG, G AM, Wolf DM, Horwitz KB.** Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. *J Biol Chem*. 2002;277:5209–5218.
242. **Mulac-Jericevic B, Conneely OM.** Reproductive tissue selective actions of progesterone receptors. *Reproduction*. 2004;128:139–146.
243. **Johnson J, Corbisier R, Stensgard B, Toft D.** The involvement of p23, hsp90, and immunophilins in the assembly of progesterone receptor complexes. *J Steroid Biochem Mol Biol*. 1996;56:31–37.
244. **Pratt WB, Toft DO.** Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocr Rev*. 1997;18:306–360.
245. **Tranguch S, Smith DF, Dey SK.** Progesterone receptor requires a co-chaperone for signalling in uterine biology and implantation. *Reprod Biomed Online 14 Spec No*. 2007;1:39–48.
246. **Brosens JJ, Hayashi N, White JO.** Progesterone receptor regulates decidual prolactin expression in differentiating human endometrial stromal cells. *Endocrinology*. 1999;140:4809–4820.
247. **Li X, O'Malley BW.** Unfolding the action of progesterone receptor. *J Biol Chem*. 2003;278:39261–39264.
248. **Large MJ, DeMayo FJ.** The regulation of embryo implantation and endometrial decidualization by progesterone receptor signaling. *Mol Cell Endocrinol*. 2012;358:155–165.
249. **Gellersen B, Fernandes MS, Brosens JJ.** Non-genomic progesterone actions in female reproduction. *Hum Reprod Update*. 2009;15:119–138.
250. **Wendler A, Albrecht C, Wehling M.** Nongenomic actions of aldosterone and progesterone revisited. *Steroids*. 2012;77:1002–1006.
251. **Lösel RM, Besong D, Peluso JJ, Wehling M.** Progesterone receptor membrane component 1- Many tasks for a versatile protein. *Steroids*. 2008;73:929–934.
252. **Pru JK, Clark N.** PGRMC1 and PGRMC2 in uterine physiology and disease. *Front Neurosci*. 2013;7:168.
253. **Zhu Y, Bond J, Thomas P.** Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progestin receptor. *Proc Natl Acad Sci USA*. 2003;100:2237–2242.
254. **Zhu Y, Hanna RN, Schaaf MJ, Spaink HP, Thomas P.**

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

Candidates for membrane progestin receptors: past approaches and future challenges. *Comp Biochem Physiol C Toxicol Pharmacol*. 2008;148:381–389.

255. **Zhang L, Kanda Y, Roberts DJ, Ecker JL, Losel R, Wehling M, Peluso JJ, Pru JK.** Expression of progesterone receptor membrane component 1 and its partner serpine 1 mRNA binding protein in uterine and placental tissues of the mouse and human. *Mol Cell Endocrinol*. 2008;287:81–89.
256. **Chen JI, Hannan NJ, Mak Y, Nicholls PK, Zhang J, Rainczuk A, Stanton PG, Robertson DM, Salamonsen LA, Stephens AN.** Proteomic characterization of midproliferative and midsecretory human endometrium. *J Proteome Res*. 2009;8:2032–2044.
257. **Bunch K, Tinnemore D, Huff S, Hoffer ZS, Burney RO, Stallings JD.** Expression patterns of progesterone receptor membrane components 1 and 2 in endometria from women with and without endometriosis. *Reprod Sci* 2013
258. **Fernandes MS, Pierron V, Michalovich D, Astle S, Thornton S, Peltoketo H, Lam EWF, Gellersen B, Huhtaniemi I, Allen J, Brosens JJ.** Regulated expression of putative membrane progestin receptor homologues in human endometrium and gestational tissues. *J Endocrinol*. 2005;187:89–101.
259. **Krietsch T, Fernandes MS, Kero J, Lösel R, Heyens M, Lam EW, Huhtaniemi I, Brosens JJ, Gellersen B.** Human homologs of the putative G protein-coupled membrane progestin receptors (mPRα, β, and γ) localize to the endoplasmic reticulum and are not activated by progesterone. *Mol Endocrinol*. 2006;20:3146–3164.
260. **Fernandes MS, Brosens JJ, Gellersen B.** Honey, we need to talk about the membrane progestin receptors. *Steroids*. 2008;73:942–952.
261. **Ahmed IS, Chamberlain C, Craven RJ.** S2R (Pgrmc1): the cytochrome-related sigma-2 receptor that regulates lipid and drug metabolism and hormone signaling. *Expert Opin Drug Metab Toxicol*. 2012;8:361–370.
262. **Mir SU, Schwarze S, Jin L, Zhang J, Friend W, Miriyala S, St Clair D, Craven RJ.** Progesterone receptor membrane component 1/Sigma-2 receptor associates with MAP1LC3B and promotes autophagy. *Autophagy*. 2013;9:1566–1578.
263. **Boonyaratanakornkit V, Edwards DP.** Receptor mechanisms mediating non-genomic actions of sex steroids. *Semin Reprod Med*. 2007;25:139–153.
264. **Vallejo G, Ballaré C, Barañao JL, Beato M, Saragüeta P.** Progestin activation of nongenomic pathways via cross talk of progesterone receptor with estrogen receptor β induces proliferation of endometrial stromal cells. *Mol Endocrinol*. 2005;19:3023–3037.
265. **Mote PA, Balleine RL, McGowan EM, Clarke CL.** Colocalization of progesterone receptors A and B by dual immunofluorescent histochemistry in human endometrium during the menstrual cycle. *J Clin Endocrinol Metab*. 1999;84:2963–2971.
266. **Gao J, Mazella J, Tang M, Tseng L.** Ligand-activated progesterone receptor isoform hPR-A is a stronger transactivator than hPR-B for the expression of IGFBP-1 (insulin-like growth factor binding protein-1) in human endometrial stromal cells. *Mol Endocrinol*. 2000;14:1954–1961.
267. **Christian M, Pohnke Y, Kempf R, Gellersen B, Brosens JJ.** Functional association of PR and CCAAT/enhancer-binding protein β isoforms: Promoter-dependent cooperation between PR-B and liver-enriched inhibitory protein, or liver-enriched activatory protein and PR-A in human endometrial stromal cells. *Mol Endocrinol*. 2002;16:141–154.
268. **Tseng L, Zhu HH.** Regulation of progesterone receptor messenger ribonucleic acid by progestin in human endometrial stromal cells. *Biol Reprod*. 1997;57:1360–1366.
269. **Curtis SW, Clark J, Myers P, Korach KS.** Disruption of estrogen signaling does not prevent progesterone action in the estrogen receptor α knockout mouse uterus. *Proc Nat Acad Sci USA*. 1999;96:3646–3651.
270. **Classen-Linke I, Alfer J, Hey S, Krusche CA, Kusche M, Beier HM.** Marker molecules of human endometrial differentiation can be hormonally regulated under in-vitro conditions as in-vivo. *Hum Reprod Update*. 1998;4:539–549.
271. **Yang S, Fang Z, Gurates B, Tamura M, Miller J, Ferrer K, Bulun SE.** Stromal PRs mediate induction of 17β-hydroxysteroid dehydrogenase type 2 expression in human endometrial epithelium: a paracrine mechanism for inactivation of E2. *Mol Endocrinol*. 2001;15:2093–2105.
272. **Janowski BA, Corey DR.** Minireview: Switching on progesterone receptor expression with duplex RNA. *Mol Endocrinol*. 2010;24:2243–2252.
273. **Chu Y, Kalantari R, Dodd DW, Corey DR.** Transcriptional silencing by hairpin RNAs complementary to a gene promoter. *Nucleic Acid Ther*. 2012;22:147–151.
274. **Panda H, Chuang TD, Luo X, Chegini N.** Endometrial miR-181a and miR-98 expression is altered during transition from normal into cancerous state and target PGR, PGRMC1, CYP19A1, DDX3X, and TIMP3. *J Clin Endocrinol Metab*. 2012;97:E1316–1326.
275. **Williams KC, Renthal NE, Condon JC, Gerard RD, Mendelson CR.** MicroRNA-200a serves a key role in the decline of progesterone receptor function leading to term and preterm labor. *Proc Natl Acad Sci USA*. 2012;109:7529–7534.
276. **Lam EW, Shah K, Brosens JJ.** The diversity of sex steroid action: the role of micro-RNAs and FOXO transcription factors in cycling endometrium and cancer. *J Endocrinol*. 2012;212:13–25.
277. **Daikoku T, Tranguch S, Friedman DB, Das SK, Smith DF, Dey SK.** Proteomic analysis identifies immunophilin FK506 binding protein 4 (FKBP52) as a downstream target of Hoxa10 in the periimplantation mouse uterus. *Mol Endocrinol*. 2005;19:683–697.
278. **Tranguch S, Cheung-Flynn J, Daikoku T, Prapapanich V, Cox MB, Xie H, Wang H, Das SK, Smith DF, Dey SK.** Cochaperone immunophilin FKBP52 is critical to uterine receptivity for embryo implantation. *Proc Natl Acad Sci USA*. 2005;102:14326–14331.
279. **Mukherjee A, Amato P, Allred DC, Fernandez-Valdivia R, Nguyen J, O'Malley BW, DeMayo FJ, Lydon JP.** Steroid receptor coactivator 2 is essential for progesterone-dependent uterine function and mammary morphogenesis: insights from the mouse – implications for the human. *J Steroid Biochem Mol Biol*. 2006;102:22–31.
280. **Park S, Yoon S, Zhao Y, Park SE, Liao L, Xu J, Lydon JP, DeMayo FJ, O'Malley BW, Bagchi MK, Katzenellenbogen**

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

BS. Uterine development and fertility are dependent on gene dosage of the nuclear receptor coregulator REA. *Endocrinology*. 2012;153:3982–3994.
281. **Zhao Y, Park S, Bagchi MK, Taylor RN, Katzenellenbogen BS.** The coregulator, repressor of estrogen receptor activity (REA), is a crucial regulator of the timing and magnitude of uterine decidualization. *Endocrinology*. 2013;154: 1349–1360.
282. **Aghajanova L, Tatsumi K, Horcajadas JA, Zamah AM, Esteban FJ, Herndon CN, Conti M, Giudice LC.** Unique transcriptome, pathways, and networks in the human endometrial fibroblast response to progesterone in endometriosis. *Biol Reprod*. 2011;84:801–815.
283. **Skålhegg BS, Taskén K.** Specificity in the cAMP/PKA signaling pathway, differential expression, regulation, and subcellular localization of subunits of PKA. *Front Biosci*. 2000;5:d678–693.
284. **Kopperud R, Krakstad C, Selheim F, Døskeland SO.** cAMP effector mechanisms. Novel twists for an 'old' signaling system. *FEBS Lett*. 2003;546:121–126.
285. **Mayr B, Montminy M.** Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat Rev Mol Cell Biol*. 2001;2:599–609.
286. **Lamas M, Monaco L, Zazopoulos E, Lalli E, Tamai K, Penna L, Mazzucchelli C, Nantel F, Foulkes NS, Sassone-Corsi P.** CREM: A master-switch in the transcriptional response to cAMP. *Philos Trans R Soc Lond, B, Biol Sci*. 1996;351:561–567.
287. **Luscombe NM, Austin SE, Berman HM, Thornton JM.** An overview of the structures of protein-DNA complexes. *Genome Biol* 2000;1:reviews001.001–001.037.
288. **Montminy M.** Transcriptional activation. Something new to hang your HAT on. *Nature*. 1997;387:654–655.
289. **de Rooij J, Zwartkruis FJT, Verheijen MHG, Cool RH, Nijman SMB, Wittinghofer A, Bos JL.** Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature*. 1998;396:474–477.
290. **Schmidt M, Dekker FJ, Maarsingh H.** Exchange protein directly activated by cAMP (epac): a multidomain cAMP mediator in the regulation of diverse biological functions. *Pharmacol Rev*. 2013;65:670–709.
291. **Mehats C, Andersen CB, Filopanti M, Jin SL, Conti M.** Cyclic nucleotide phosphodiesterases and their role in endocrine cell signaling. *Trends Endocrinol Metab*. 2002;13: 29–35.
292. **Tanaka N, Miyazaki K, Tashiro H, Mizutani H, Okamura H.** Changes in adenylyl cyclase activity in human endometrium during the menstrual cycle and in human decidua during pregnancy. *J Reprod Fertil*. 1993;98:33–39.
293. **Bergamini CM, Pansini F, Bettocchi Jr. S, Segala V, Dallocchio F, Bagni B, Mollica G.** Hormonal sensitivity of adenylate cyclase from human endometrium: modulation by estradiol. *J Steroid Biochem*. 1985;22:299–303.
294. **Yoshino O, Osuga Y, Hirota Y, Koga K, Yano T, Tsutsumi O, Taketani Y.** Akt as a possible intracellular mediator for decidualization in human endometrial stromal cells. *Mol Hum Reprod*. 2003;9:265–269.
295. **Brar AK, Frank GR, Kessler CA, Cedars MI, Handwerger S.** Progesterone-dependent decidualization of the human endometrium is mediated by cAMP. *Endocrine*. 1997;6: 301–307.
296. **Houserman VL, Todd H, Hertelendy F.** Progesterone treatment *in vitro* enhances prostaglandin E and forskolin-promoted cyclic AMP production in human endometrial stromal cells. *J Reprod Fertil*. 1989;85:195–202.
297. **Telgmann R, Maronde E, Taskén K, Gellersen B.** Activated protein kinase A is required for differentiation-dependent transcription of the decidual prolactin gene in human endometrial stromal cells. *Endocrinology*. 1997;138: 929–937.
298. **Telgmann R, Gellersen B.** Marker genes of decidualization: activation of the decidual prolactin gene. *Hum Reprod Update*. 1998;4:472–479.
299. **Bartsch O, Bartlick B, Ivell R.** Phosphodiesterase 4 inhibition synergizes with relaxin signaling to promote decidualization of human endometrial stromal cells. *J Clin Endocrinol Metab*. 2004;89:324–334.
300. **Bartsch O, Ivell R.** Relaxin and phosphodiesterases collaborate during decidualization. *Ann N Y Acad Sci*. 2004; 1030:479–492.
301. **Kusama K, Yoshie M, Tamura K, Kodaka Y, Hirata A, Sakurai T, Bai H, Imakawa K, Nishi H, Isaka K, Nagai T, Nagao T, Tachikawa E.** Regulation of decidualization in human endometrial stromal cells through exchange protein directly activated by cyclic AMP (Epac). *Placenta*. 2013;34:212–221.
302. **Gellersen B, Kempf R, Telgmann R.** Human endometrial stromal cells express novel isoforms of the transcriptional modulator CREM and up-regulate ICER in the course of decidualization. *Mol Endocrinol*. 1997;11:97–113.
303. **Molina CA, Foulkes NS, Lalli E, Sassone-Corsi P.** Inducibility and negative autoregulation of CREM: an alternative promoter directs the expression of ICER, an early response repressor. *Cell*. 1993;75:875–886.
304. **Gellersen B, DiMattia GE, Friesen HG, Bohnet HG.** Prolactin (PRL) mRNA from human decidua differs from pituitary PRL mRNA but resembles the IM-9-P3 lymphoblast PRL transcript. *Mol Cell Endocrinol*. 1989;64:127–130.
305. **DiMattia GE, Gellersen B, Duckworth ML, Friesen HG.** Human prolactin gene expression. The use of an alternative noncoding exon in decidua and the IM-9-P3 lymphoblast cell line. *J Biol Chem*. 1990;265:16412–16421.
306. **Gellersen B, Kempf R, Telgmann R, DiMattia GE.** Non-pituitary human prolactin gene transcription is independent of Pit-1 and differentially controlled in lymphocytes and in endometrial stroma. *Mol Endocrinol*. 1994;8:356–373.
307. **Berwaer M, Martial JA, Davis JRE.** Characterization of an up-stream promoter directing extrapituitary expression of the human prolactin gene. *Mol Endocrinol*. 1994;8:635–642.
308. **Golander A, Barrett J, Hurley T, Barry S, Handwerger S.** Failure of bromocriptine, dopamine, and thyrotropin-releasing hormone to affect prolactin secretion by human decidual tissue in vitro. *J Clin Endocrinol Metab*. 1979; 49:787–789.
309. **Al-Sabbagh M, Fusi L, Higham J, Lee Y, Lei K, Hanyaloglu AC, Lam EW, Christian M, Brosens JJ.** NADPH oxidase-derived reactive oxygen species mediate decidualization of human endometrial stromal cells in response to cyclic AMP signaling. *Endocrinology*. 2011;152:730–740.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

310. **Fedoroff NV.** Presidential address. Transposable elements, epigenetics, and genome evolution. *Science*. 2012;338:758–767.
311. **Wicker T, Sabot F, Hua-Van A, Bennetzen JL, Capy P, Chalhoub B, Flavell A, Leroy P, Morgante M, Panaud O, Paux E, SanMiguel P, Schulman AH.** A unified classification system for eukaryotic transposable elements. *Nat Rev Genet*. 2007;8:973–982.
312. **Gerlo S, Davis JR, Mager DL, Kooijman R.** Prolactin in man: a tale of two promoters. *Bioessays*. 2006;28:1051–1055.
313. **Lynch VJ, Leclerc RD, May G, Wagner GP.** Transposon-mediated rewiring of gene regulatory networks contributed to the evolution of pregnancy in mammals. *Nat Genet*. 2011;43:1154–1159.
314. **Wagner GP, Lynch VJ.** Evolutionary novelties. *Curr Biol*. 2010;20:R48–52.
315. **Emera D, Casola C, Lynch VJ, Wildman DE, Agnew D, Wagner GP.** Convergent evolution of endometrial prolactin expression in primates, mice, and elephants through the independent recruitment of transposable elements. *Mol Biol Evol*. 2012;29:239–247.
316. **Emera D, Wagner GP.** Transformation of a transposon into a derived prolactin promoter with function during human pregnancy. *Proc Natl Acad Sci USA*. 2012;109:11246–11251.
317. **Lekstrom-Himes J, Xanthopoulos KG.** Biological role of the CCAAT/enhancer-binding protein family of transcription factors. *J Biol Chem*. 1998;273:28545–28548.
318. **Pohnke Y, Kempf R, Gellersen B.** CCAAT/enhancer-binding proteins are mediators in the protein kinase A-dependent activation of the decidual prolactin gene. *J Biol Chem*. 1999;274:24808–24818.
319. **Buggisch M, Ateghang B, Ruhe C, Strobel C, Lange S, Wartenberg M, Sauer H.** Stimulation of ES-cell-derived cardiomyogenesis and neonatal cardiac cell proliferation by reactive oxygen species and NADPH oxidase. *J Cell Sci*. 2007;120:885–894.
320. **Clempus RE, Sorescu D, Dikalova AE, Pounkova L, Jo P, Sorescu GP, Schmidt HH, Lassegue B, Griendling KK.** Nox4 is required for maintenance of the differentiated vascular smooth muscle cell phenotype. *Arterioscler Thromb Vasc Biol*. 2007;27:42–48.
321. **Schröder K, Wandzioch K, Helmcke I, Brandes RP.** Nox4 acts as a switch between differentiation and proliferation in preadipocytes. *Arterioscler Thromb Vasc Biol*. 2009;29:239–245.
322. **Plante BJ, Kannan A, Bagchi MK, Yuan L, Young SL.** Cyclic regulation of transcription factor C/EBP beta in human endometrium. *Reprod Biol Endocrinol*. 2009;7:15.
323. **Wang W, Taylor RN, Bagchi IC, Bagchi MK.** Regulation of human endometrial stromal proliferation and differentiation by C/EBPβ involves cyclin E-cdk2 and STAT3. *Mol Endocrinol*. 2012;26:2016–2030.
324. **Mantena SR, Kannan A, Cheon YP, Li Q, Johnson PF, Bagchi IC, Bagchi MK.** C/EBPβ is a critical mediator of steroid hormone-regulated cell proliferation and differentiation in the uterine epithelium and stroma. *Proc Natl Acad Sci USA*. 2006;103:1870–1875.
325. **Lalmansingh AS, Karmakar S, Jin Y, Nagaich AK.** Multiple modes of chromatin remodeling by Forkhead box proteins. *Biochim Biophys Acta*. 2012;1819:707–715.
326. **Burgering BM, Kops GJ.** Cell cycle and death control: long live Forkheads. *Trends Biochem Sci*. 2002;27:352–360.
327. **Tran H, Brunet A, Griffith EC, Greenberg ME.** The many forks in FOXO's road. *Sci STKE* 2003;172:re5.
328. **Accili D, Arden KC.** FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. *Cell*. 2004;117:421–426.
329. **Nemoto S, Fergusson MM, Finkel T.** Nutrient availability regulates SIRT1 through a Forkhead-dependent pathway. *Science*. 2004;306:2105–2108.
330. **Lam EW, Brosens JJ, Gomes AR, Koo CY.** Forkhead box proteins: tuning forks for transcriptional harmony. *Nat Rev Cancer*. 2013;13:482–495.
331. **Brunet A, Park J, Tran H, Hu LS, Hemmings BA, Greenberg ME.** Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHRL1 (FOXO3a). *Mol Cell Biol*. 2001;21:952–965.
332. **Feroze-Zaidi F, Fusi L, Takano M, Higham J, Salker MS, Goto T, Edassery S, Klingel K, Boini KM, Palmada M, Kamps R, Groothuis PG, Lam EW, Smith SK, Lang F, Sharkey AM, Brosens JJ.** Role and regulation of the serum- and glucocorticoid-regulated kinase 1 in fertile and infertile human endometrium. *Endocrinology*. 2007;148:5020–5029.
333. **Essers MA, Weijzen S, de Vries-Smits AM, Saarloos I, de Ruiter ND, Bos JL, Burgering BM.** FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. *EMBO J*. 2004;23:4802–4812.
334. **Labied S, Kajihara T, Madureira PA, Fusi L, Jones MC, Higham JM, Varshochi R, Francis JM, Zoumpoulidou G, Essafi A, Fernandez de Mattos S, Lam EW, Brosens JJ.** Progestins regulate the expression and activity of the Forkhead transcription factor FOXO1 in differentiating human endometrium. *Mol Endocrinol*. 2006;20:35–44.
335. **Sekiya T, Adachi S, Kohu K, Yamada T, Higuchi O, Furukawa Y, Nakamura Y, Nakamura T, Tashiro K, Kuhara S, Ohwada S, Akiyama T.** Identification of BMP and activin membrane-bound inhibitor (BAMBI), an inhibitor of transforming growth factor β signaling, as a target of the β-catenin pathway in colorectal tumor cells. *J Biol Chem*. 2004;279:6840–6846.
336. **Schroeder JK, Kessler CA, Handwerger S.** Critical role for TWIST1 in the induction of human uterine decidualization. *Endocrinology*. 2011;152:4368–4376.
337. **Grinius L, Kessler CA, Schroeder J, Handwerger S.** Forkhead transcription factor FOXO1A is critical for induction of human decidualization. *J Endocrinol*. 2006;189:179–187.
338. **Kessler CA, Schroeder JK, Brar AK, Handwerger S.** Transcription factor ETS1 is critical for human uterine decidualization. *Mol Hum Reprod*. 2006;12:71–76.
339. **Brosens JJ, Wilson MS, Lam EW.** FOXO transcription factors: from cell fate decisions to regulation of human female reproduction. *Adv Exp Med Biol*. 2009;665:227–241.
340. **Kim JJ, Buzzio OL, Li S, Lu Z.** Role of FOXO1A in the regulation of insulin-like growth factor binding protein-1 in human endometrial cells: Interaction with progesterone receptor. *Biol Reprod*. 2005;73:833–839.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

341. **Brosens JJ, Gellersen B.** Death or survival - progesterone-dependent cell fate decisions in the human endometrial stroma. *J Mol Endocrinol*. 2006;36:389–398.
342. **Favier B, Dollé P.** Developmental functions of mammalian *Hox* genes. *Mol Hum Reprod*. 1997;3:115–131.
343. **Taylor HS, Vanden Heuvel GB, Igarashi P.** A conserved *Hox* axis in the mouse and human female reproductive system: late establishment and persistent adult expression of the *Hoxa* cluster genes. *Biol Reprod*. 1997;57:1338–1345.
344. **Hsieh-Li HM, Witte DP, Weinstein M, Branford W, Li H, Small K, Potter SS.** *Hoxa* 11 structure, extensive antisense transcription, and function in male and female fertility. *Development*. 1995;121:1373–1385.
345. **Satokata I, Benson G, Maas R.** Sexually dimorphic sterility phenotypes in *Hoxa10*-deficient mice. *Nature*. 1995;374:460–463.
346. **Gendron RL, Paradis H, Hsieh-Li HM, Lee DW, Potter SS, Markoff E.** Abnormal uterine stromal and glandular function associated with maternal reproductive defects in Hoxa-11 null mice. *Biol Reprod*. 1997;56:1097–1105.
347. **Lim HJ, Ma L, Ma WG, Mass RL, Dey SK.** Hoxa-10 regulates uterine stromal cell responsiveness to progesterone during implantation and decidualization in the mouse. *Mol Endocrinol*. 1999;13:1005–1017.
348. **Kwon YW, Ueda S, Ueno M, Yodoi J, Masutani H.** Mechanism of p53-dependent apoptosis induced by 3-methylcholanthrene: involvement of p53 phosphorylation and p38 MAPK. *J Biol Chem*. 2002;277:1837–1844.
349. **Taylor HS, Igarashi P, Olive DL, Arici A.** Sex steroids mediate HOXA11 expression in the human peri-implantation endometrium. *J Clin Endocrinol Metab*. 1999;84:1129–1135.
350. **Taylor HS, Arici A, Olive D, Igarashi P.** HOXA10 is expressed in response to sex steroids at the time of implantation in the human endometrium. *J Clin Invest*. 1998;101:1379–1384.
351. **Gui YT, Zhang JN, Yuan LW, Lessey BA.** Regulation of HOXA-10 and its expression in normal and abnormal endometrium. *Mol Hum Reprod*. 1999;5:866–873.
352. **Kwon HE, Taylor HS.** The role of *HOX* genes in human implantation. *Ann N Y Acad Sci*. 2004;1034:1–18.
353. **Yang Z, Wolf IM, Chen H, Periyasamy S, Chen Z, Yong W, Shi S, Zhao W, Xu J, Srivastava A, Sanchez ER, Shou W.** FK506-binding protein 52 Is essential to uterine reproductive physiology controlled by the progesterone receptor A isoform. *Mol Endocrinol*. 2006;20:2682–2694.
354. **Lynch VJ, Brayer K, Gellersen B, Wagner GP.** HoxA-11 and FOXO1A cooperate to regulate decidual prolactin expression: Towards inferring the core transcriptional regulators of decidual genes. *PLoS ONE*. 2009;4:e6845.
355. **Gennari-Moser C, Khankin EV, Schuller S, Escher G, Frey BM, Portmann CB, Baumann MU, Lehmann AD, Surbek D, Karumanchi SA, Frey FJ, Mohaupt MG.** Regulation of placental growth by aldosterone and cortisol. *Endocrinology*. 2011;152:263–271.
356. **Castracane VD, Stewart DR, Gimpel T, Overstreet JW, Lasley BL.** Maternal serum androgens in human pregnancy: early increases within the cycle of conception. *Hum Reprod*. 1998;13:460–464.
357. **Jensen E, Wood C, Keller-Wood M.** The normal increase in adrenal secretion during pregnancy contributes to maternal volume expansion and fetal homeostasis. *J Soc Gynecol Invest*. 2002;9:362–371.
358. **Bonney RC, Scanlon MJ, Jones DL, Reed MJ, James VH.** Adrenal androgen concentrations in endometrium and plasma during the menstrual cycle. *J Endocrinol*. 1984;101:181–188.
359. **Vermeulen-Meiners C, Poortman J, Nabuurs M, Thijssen JH.** The endogenous concentration and subcellular distribution of androgens in normal human premenopausal endometrium, myometrium and vagina. *Gynecol Endocrinol*. 1988;2:121–130.
360. **Mertens HJ, Heineman MJ, Theunissen PH, de Jong FH, Evers JL.** Androgen, estrogen and progesterone receptor expression in the human uterus during the menstrual cycle. *Eur J Obstet Gynecol Reprod Biol*. 2001;98:58–65.
361. **Cloke B, Shah K, Kaneda H, Lavery S, Trew G, Fusi L, Higham J, Dina RE, Ghaem-Maghami S, Ellis P, Brosens JJ, Christian M.** The poly(c) -binding protein-1 regulates expression of the androgen receptor. *Endocrinology* 2010;151:3954–3964.
362. **Slayden OD, Nayak NR, Burton KA, Chwalisz K, Cameron ST, Critchley HO, Baird DT, Brenner RM.** Progesterone antagonists increase androgen receptor expression in the rhesus macaque and human endometrium. *J Clin Endocrinol Metab*. 2001;86:2668–2679.
363. **Kemppainen JA, Wilson EM.** Agonist and antagonist activities of hydroxyflutamide and Casodex relate to androgen receptor stabilization. *Urology*. 1996;48:157–163.
364. **Henderson TA, Saunders PT, Moffett-King A, Groome NP, Critchley HO.** Steroid receptor expression in uterine natural killer cells. *J Clin Endocrinol Metab*. 2003;88:440–449.
365. **McDonald SE, Henderson TA, Gomez-Sanchez CE, Critchley HO, Mason JI.** 11β-hydroxysteroid dehydrogenases in human endometrium. *Mol Cell Endocrinol*. 2006;248:72–78.
366. **Funder JW.** Glucocorticoid and mineralocorticoid receptors: biology and clinical relevance. *Annu Rev Med*. 1997;48:231–240.
367. **Myles K, Funder JW.** Progesterone binding to mineralocorticoid receptors: in vitro and in vivo studies. *Am J Physiol*. 1996;270:E601–607.
368. **Selman PJ, Wolfswinkel J, Mol JA.** Binding specificity of medroxyprogesterone acetate and proligestone for the progesterone and glucocorticoid receptor in the dog. *Steroids*. 1996;61:133–137.
369. **Schindler AE, Campagnoli C, Druckmann R, Huber J, Pasqualini JR, Schweppe KW, Thijssen JH.** Classification and pharmacology of progestins. *Maturitas*. 2008;61:171–180.
370. **Chandrasekhar Y, Armstrong DT, Kennedy TG.** Implantation delay and anti-deciduogenic activity in the rat by the anti-androgen, hydroxyflutamide. *Biol Reprod*. 1990;42:120–125.
371. **Groner AC, Meylan S, Ciuffi A, Zangger N, Ambrosini G, Denervaud N, Bucher P, Trono D.** KRAB-zinc finger proteins and KAP1 can mediate long-range transcriptional repression through heterochromatin spreading. *PLoS Genet* 2010;6:e1000869.
372. **Burris TP, Busby SA, Griffin PR.** Targeting orphan nuclear

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

receptors for treatment of metabolic diseases and autoimmunity. *Chem Biol*. 2012;19:51–59.
373. **Lee YK, Moore DD.** Liver receptor homolog-1, an emerging metabolic modulator. *Front Biosci*. 2008;13:5950–5958.
374. **Mouzat K, Baron S, Marceau G, Caira F, Sapin V, Volle DH, Lumbroso S, Lobaccaro JM.** Emerging roles for LXRs and LRH-1 in female reproduction. *Mol Cell Endocrinol*. 2013;368:47–58.
375. **Zhang C, Large MJ, Duggavathi R, DeMayo FJ, Lydon JP, Schoonjans K, Kovanci E, Murphy BD.** Liver receptor homolog-1 is essential for pregnancy. *Nat Med*. 2013;19:1061–1066.
376. **Lin FJ, Qin J, Tang K, Tsai SY, Tsai MJ.** Coup d'Etat: an orphan takes control. *Endocr Rev*. 2011;32:404–421.
377. **Kurihara I, Lee DK, Petit FG, Jeong J, Lee K, Lydon JP, DeMayo FJ, Tsai MJ, Tsai SY.** COUP-TFII mediates progesterone regulation of uterine implantation by controlling ER activity. *PLoS Genet*. 2007;3:e102.
378. **Takamoto N, Kurihara I, Lee K, Demayo FJ, Tsai MJ, Tsai SY.** Haploinsufficiency of chicken ovalbumin upstream promoter transcription factor II in female reproduction. *Mol Endocrinol*. 2005;19:2299–2308.
379. **Lee DK, Kurihara I, Jeong JW, Lydon JP, DeMayo FJ, Tsai MJ, Tsai SY.** Suppression of ERα activity by COUP-TFII is essential for successful implantation and decidualization. *Mol Endocrinol*. 2010;24:930–940.
380. **Li X, Large MJ, Creighton CJ, Lanz RB, Jeong JW, Young SL, Lessey BA, Palomino WA, Tsai SY, Demayo FJ.** COUP-TFII regulates human endometrial stromal genes involved in inflammation. *Mol Endocrinol*. 2013;27:2041–2054.
381. **Krishnan V, Pereira FA, Qiu Y, Chen CH, Beachy PA, Tsai SY, Tsai MJ.** Mediation of Sonic hedgehog-induced expression of COUP-TFII by a protein phosphatase. *Science*. 1997;278:1947–1950.
382. **Bombail V, Gibson DA, Collins F, MacPherson S, Critchley HO, Saunders PT.** A role for the orphan nuclear receptor estrogen-related receptor α in endometrial stromal cell decidualization and expression of genes implicated in energy metabolism. *J Clin Endocrinol Metab*. 2010;95:E224–228.
383. **Jiang Y, Hu Y, Zhao J, Zhen X, Yan G, Sun H.** The orphan nuclear receptor Nur77 regulates decidual prolactin expression in human endometrial stromal cells. *Biochem Biophys Res Commun*. 2011;404:628–633.
384. **Goddard LM, Murphy TJ, Org T, Enciso JM, Hashimoto-Partyka MK, Warren CM, Domigan CK, McDonald AI, He H, Sanchez LA, Allen NC, Orsenigo F, Chao LC, Dejana E, Tontonoz P, Mikkola HK, Iruela-Arispe ML.** Progesterone receptor in the vascular endothelium triggers physiological uterine permeability preimplantation. *Cell*. 2014;156:549–562.
385. **Shuai K, Liu B.** Regulation of JAK-STAT signalling in the immune system. *Nat Rev Immunol*. 2003;3:900–911.
386. **Dimitriadis E, Stoikos C, Tan YL, Salamonsen LA.** Interleukin 11 signaling components signal transducer and activator of transcription 3 (STAT3) and suppressor of cytokine signaling 3 (SOCS3) regulate human endometrial stromal cell differentiation. *Endocrinology*. 2006;147:3809–3817.
387. **Mak IYH, Brosens JJ, Christian M, Hills FA, Chamley L, Regan L, White JO.** Regulated expression of signal transducer and activator of transcription, Stat5, and its enhancement of PRL expression in human endometrial stromal cells *in vitro*. *J Clin Endocrinol Metab*. 2002;87:2581–2588.
388. **Kudo Y, Hara T, Katsuki T, Toyofuku A, Katsura Y, Takikawa O, Fujii T, Ohama K.** Mechanisms regulating the expression of indoleamine 2,3-dioxygenase during decidualization of human endometrium. *Hum Reprod*. 2004;19:1222–1230.
389. **Miller LD, Smeds J, George J, Vega VB, Vergara L, Ploner A, Pawitan Y, Hall P, Klaar S, Liu ET, Bergh J.** An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc Natl Acad Sci USA*. 2005;102:13550–13555.
390. **Mirza A, Wu Q, Wang L, McClanahan T, Bishop WR, Gheyas F, Ding W, Hutchins B, Hockenberry T, Kirschmeier P, Greene JR, Liu S.** Global transcriptional program of p53 target genes during the process of apoptosis and cell cycle progression. *Oncogene*. 2003;22:3645–3654.
391. **Ryan KM, Phillips AC, Vousden KH.** Regulation and function of the p53 tumor suppressor protein. *Curr Opin Cell Biol*. 2001;13:332–337.
392. **Vogelstein B, Lane D, Levine AJ.** Surfing the p53 network. *Nature*. 2000;408:307–310.
393. **Hu W, Feng Z, Teresky AK, Levine AJ.** p53 regulates maternal reproduction through LIF. *Nature*. 2007;450:721–724.
394. **Logan PC, Steiner M, Ponnampalam AP, Mitchell MD.** Cell cycle regulation of human endometrial stromal cells during decidualization. *Reprod Sci*. 2012;19:883–894.
395. **Taylor WR, Stark GR.** Regulation of the G2/M transition by p53. *Oncogene*. 2001;20:1803–1815.
396. **Borthwick JM, Charnock-Jones DS, Tom BD, Hull ML, Teirny R, Phillips SC, Smith SK.** Determination of the transcript profile of human endometrium. *Mol Hum Reprod*. 2003;9:19–33.
397. **Mirkin S, Arslan M, Churikov D, Corica A, Diaz JI, Williams S, Bocca S, Oehninger S.** In search of candidate genes critically expressed in the human endometrium during the window of implantation. *Hum Reprod*. 2005;20:2104–2117.
398. **Kajihara T, Jones M, Fusi L, Takano M, Feroze-Zaidi F, Pirianov G, Mehmet H, Ishihara O, Higham JM, Lam EW, Brosens JJ.** Differential expression of FOXO1 and FOXO3a confers resistance to oxidative cell death upon endometrial decidualization. *Mol Endocrinol*. 2006;20:2444–2455.
399. **Zerbini LF, Libermann TA.** Life and death in cancer. GADD45α and γ are critical regulators of NF-κB mediated escape from programmed cell death. *Cell Cycle*. 2005;4:18–20.
400. **Burton GJ, Jauniaux E.** Placental oxidative stress: from miscarriage to preeclampsia. *J Soc Gynecol Invest*. 2004;11:342–352.
401. **You H, Mak TW.** Crosstalk between p53 and FOXO transcription factors. *Cell Cycle*. 2005;4:37–38.
402. **Fahnenstich J, Nandy A, Milde-Langosch K, Schneider-**

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

Merck T, Walther N, Gellersen B. Promyelocytic leukemica zinc finger protein (PLZF) is a glucocorticoid- and progesterone-regulated transcription factor in human endometrial stromal cells and myometrial smooth muscle cells. *Mol Hum Reprod*. 2003;9:611–623.

403. **Suliman BA, Xu D, Williams BR.** The promyelocytic leukemia zinc finger protein: two decades of molecular oncology. *Front Oncol*. 2012;2:74.
404. **Costoya JA, Pandolfi PP.** The role of promyelocytic leukemia zinc finger and promyelocytic leukemia in leukemogenesis and development. *Curr Opin Hematol*. 2001;8: 212–217.
405. **Melnick A, Ahmad KF, Arai S, Polinger A, Ball H, Borden KL, Carlile GW, Prive GG, Licht JD.** In-depth mutational analysis of the promyelocytic leukemia zinc finger BTB/POZ domain reveals motifs and residues required for biological and transcriptional functions. *Mol Cell Biol*. 2000;20:6550–6567.
406. **Yeyati PL, Shaknovich R, Boterashvili S, Li J, Ball HJ, Waxman S, Nason-Burchenal K, Dmitrovsky E, Zelent A, Licht JD.** Leukemia translocation protein PLZF inhibits cell growth and expression of cyclin A. *Oncogene*. 1999; 18:925–934.
407. **Parrado A, Robledo M, Moya-Quiles MR, Marín LA, Chomienne C, Padua RA, Álvarez-López MR.** The promyelocytic leukemia zinc finger protein down-regulates apoptosis and expression of the proapoptotic BID protein in lymphocytes. *Proc Natl Acad Sci USA*. 2004;101:1898–1903.
408. **Chinenov Y, Kerppola TK.** Close encounters of many kinds: Fos-Jun interactions that mediate transcription regulatory specificity. *Oncogene*. 2001;20:2438–2452.
409. **Salmi A, Rutanen FM.** C-fos and c-jun expression in human endometrium and myometrium. *Mol Cell Endocrinol*. 1996;117:233–240.
410. **Watanabe K, Kessler CA, Bachurski CJ, Kanda Y, Richardson BD, Stanek J, Handwerger S, Brar AK.** Identification of a decidua-specific enhancer on the human prolactin gene with two critical activator protein 1 (AP-1) binding sites. *Mol Endocrinol*. 2001;15:638–653.
411. **Wasylyk B, Hagman J, Gutierrez-Hartmann A.** Ets transcription factors: nuclear effectors of the Ras-MAP-kinase signaling pathway. *Trends Biochem Sci*. 1998;23:213–216.
412. **Suske G.** The Sp-family of transcription factors. *Gene*. 1999;238:291–300.
413. **Krikun G, Schatz F, Mackman N, Guller S, Demopoulos R, Lockwood CJ.** Regulation of tissue factor gene expression in human endometrium by transcription factors Sp1 and Sp3. *Mol Endocrinol*. 2000;14:393–400.
414. **Krikun G, Lockwood CJ.** Steroid hormones, endometrial gene regulation and the Sp1 family of proteins. *J Soc Gynecol Invest*. 2002;9:329–334.
415. **Rubel CA, Lanz RB, Kommagani R, Franco HL, Lydon JP, Demayo FJ.** Research resource: genome-wide profiling of progesterone receptor binding in the mouse uterus. *Mol Endocrinol*. 2012;26:1428–1442.
416. **Rubel CA, Heather LF, Camper S, Lanz RB, Jeong J, Lydon JP, DeMayo FJ.** Gata2 is a master regulator of endometrial function and progesterone signaling. Program of the 44th Annual Meeting of the Society for the Study of Reproduction, OR, OR, 2011 (Abstract 179). *Biol Reprod* 2011;85 (1 Supplement):179.
417. **Dyson MT, Roqueiro D, Monsivais D, Ercan CM, Pavone ME, Brooks DC, Kakinuma T, Ono M, Jafari N, Dai Y, Bulun SE.** Genome-wide DNA methylation analysis predicts an epigenetic switch for GATA factor expression in endometriosis. *PLoS Genet* 2014;10:e1004158.
418. **Munro SK, Farquhar CM, Mitchell MD, Ponnampalam AP.** Epigenetic regulation of endometrium during the menstrual cycle. *Mol Hum Reprod*. 2010;16:297–310.
419. **Zelenko Z, Aghajanova L, Irwin JC, Giudice LC.** Nuclear receptor, coregulator signaling, and chromatin remodeling pathways suggest involvement of the epigenome in the steroid hormone response of endometrium and abnormalities in endometriosis. *Reprod Sci*. 2012;19:152–162.
420. **Grimaldi G, Christian M, Quenby S, Brosens JJ.** Expression of epigenetic effectors in decidualizing human endometrial stromal cells. *Mol Hum Reprod*. 2012;18:451–458.
421. **Sakai N, Maruyama T, Sakurai R, Masuda H, Yamamoto Y, Shimizu A, Kishi I, Asada H, Yamagoe S, Yoshimura Y.** Involvement of histone acetylation in ovarian steroid-induced decidualization of human endometrial stromal cells. *J Biol Chem*. 2003;278:16675–16682.
422. **Estella C, Herrer I, Atkinson SP, Quinonero A, Martinez S, Pellicer A, Simon C.** Inhibition of histone deacetylase activity in human endometrial stromal cells promotes extracellular matrix remodelling and limits embryo invasion. *PLoS ONE*. 2012;7:e30508.
423. **Logan PC, Ponnampalam AP, Rahnama F, Lobie PE, Mitchell MD.** The effect of DNA methylation inhibitor 5-Aza-2′-deoxycytidine on human endometrial stromal cells. *Hum Reprod*. 2010;25:2859–2869.
424. **Logan PC, Ponnampalam AP, Steiner M, Mitchell MD.** Effect of cyclic AMP and estrogen/progesterone on the transcription of DNA methyltransferases during the decidualization of human endometrial stromal cells. *Mol Hum Reprod*. 2013;19:302–312.
425. **Gao F, Ma X, Rusie A, Hemingway J, Ostmann AB, Chung D, Das SK.** Epigenetic changes through DNA methylation contribute to uterine stromal cell decidualization. *Endocrinology*. 2012;153:6078–6090.
426. **Thurman RE, Rynes E, Humbert R, Vierstra J, Maurano MT, Haugen E, Sheffield NC, Stergachis AB, Wang H, Vernot B, Garg K, John S, Sandstrom R, Bates D, Boatman L, Canfield TK, Diegel M, Dunn D, Ebersol AK, Frum T, Giste E, Johnson AK, Johnson EM, Kutyavin T, Lajoie B, Lee BK, Lee K, London D, Lotakis D, Neph S, Neri F, Nguyen ED, Qu H, Reynolds AP, Roach V, Safi A, Sanchez ME, Sanyal A, Shafer A, Simon JM, Song L, Vong S, Weaver M, Yan Y, Zhang Z, Zhang Z, Lenhard B, Tewari M, Dorschner MO, Hansen RS, Navas PA, Stamatoyannopoulos G, Iyer VR, Lieb JD, Sunyaev SR, Akey JM, Sabo PJ, Kaul R, Furey TS, Dekker J, Crawford GE, Stamatoyannopoulos JA.** The accessible chromatin landscape of the human genome. *Nature*. 2012;489:75–82.
427. **John S, Sabo PJ, Thurman RE, Sung MH, Biddie SC, Johnson TA, Hager GL, Stamatoyannopoulos JA.** Chromatin accessibility pre-determines glucocorticoid receptor binding patterns. *Nat Genet*. 2011;43:264–268.
428. **Yin P, Roqueiro D, Huang L, Owen JK, Xie A, Navarro A,**

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

Monsivais D, Coon JSt, Kim JJ, Dai Y, Bulun SE. Genome-wide progesterone receptor binding: cell type-specific and shared mechanisms in T47D breast cancer cells and primary leiomyoma cells. *PLoS ONE*. 2012;7:e29021.

429. **Clarke CL, Graham JD.** Non-overlapping progesterone receptor cistromes contribute to cell-specific transcriptional outcomes. *PLoS ONE*. 2012;7:e35859.
430. **Sommer EM, Dry H, Cross D, Guichard S, Davies BR, Alessi DR.** Elevated SGK1 predicts resistance of breast cancer cells to Akt inhibitors. *Biochem J*. 2013;452:499–508.
431. **Lee CH, Kim TH, Lee JH, OH SJ, Yoo JY, Kwon HS, Kim YI, Ferguson SD, Ahn JY, Ku BJ, Fazleabas AT, Lim JM, Jeong JW.** Extracellular signal-regulated kinase 1/2 signaling pathway is required for endometrial decidualization in mice and human. *PLoS ONE*. 2013;8:e75282.
432. **Maruyama T, Yoshimura Y, Yodoi J, Sabe H.** Activation of c-Src kinase is associated with *in vitro* decidualization of human endometrial stromal cells. *Endocrinology*. 1999; 140:2632–2636.
433. **Yamamoto Y, Maruyama T, Sakai N, Sakurai R, Shimizu A, Hatamani T, Masuda H, Uchida H, Sabe H, Yoshimura Y.** Expression and subcellular distribution of the active form of c-Src tyrosine kinase in differentiating human endometrial stromal cells. *Mol Hum Reprod*. 2002;8:1117–1124.
434. **Nagashima T, Maruyama T, Uchida H, Kajitani T, Arase T, Ono M, Oda H, Kagami M, Masuda H, Nishikawa S, Asada H, Yoshimura Y.** Activation of SRC kinase and phosphorylation of signal transducer and activator of transcription-5 are required for decidual transformation of human endometrial stromal cells. *Endocrinology*. 2008;149: 1227–1234.
435. **Shimizu A, Maruyama T, Tamaki K, Uchida H, Asada H, Yoshimura Y.** Impairment of decidualization in SRC-deficient mice. *Biol Reprod*. 2005;73:1219–1227.
436. **Boonyaratanakornkit V, Scott MP, Ribon V, Sherman L, Anderson SM, Maller JL, Miller WT, Edwards DP.** Progesterone receptor contains a proline-rich motif that directly interacts with SH3 domains and activates c-Src family tyrosine kinases. *Mol Cell*. 2001;8:269–280.
437. **Hay RT.** SUMO: a history of modification. *Mol Cell*. 2005;18:1–12.
438. **Jones MC, Fusi L, Higham JH, Abdel-Hafiz H, Horwitz KB, Lam EW, Brosens JJ.** Regulation of the SUMO pathway sensitizes differentiating human endometrial stromal cells to progesterone. *Proc Natl Acad Sci USA*. 2006;103: 16272–16277.
439. **Yoshinaga K 1980 Inhibition of implantation by advancement of uterine sensitivity and refractoriness (Progress in Reproductive Biology and Medicine, Vol. 7). In: Leroy F, Finn CA, Psychoyos A, Hubinont PO eds. Blastocyst-Endometrium Relationships. Basel, Switzerland: Karger; 189–199.**
440. **Yoshinaga K.** Uterine receptivity for blastocyst implantation. *Ann N Y Acad Sci*. 1988;541:424–431.
441. **Psychoyos A.** Uterine receptivity for nidation. *Ann N Y Acad Sci*. 1986;476:36–42.
442. **Norwitz ER, Schust DJ, Fisher SJ.** Implantation and the survival of early pregnancy. *N Engl J Med*. 2001;345: 1400–1408.
443. **Wang H, Dey SK.** Roadmap to embryo implantation: clues from mouse models. *Nat Rev Genet*. 2006;7:185–199.
444. **Zhang S, Lin H, Kong S, Wang S, Wang H, Wang H, Armant DR.** Physiological and molecular determinants of embryo implantation. *Mol Aspects Med*. 2013;34:939–980.
445. **Cha J, Sun X, Dey SK.** Mechanisms of implantation: strategies for successful pregnancy. *Nat Med*. 2012;18:1754–1767.
446. **McCormack JT, Greenwald GS.** Evidence for a preimplantation rise in oestradiol-17beta levels on day 4 of pregnancy in the mouse. *J Reprod Fertil*. 1974;41:297–301.
447. **Carson DD, Bagchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA, Yoshinaga K.** Embryo implantation. *Dev Biol*. 2000;223:217–237.
448. **Bergh PA, Navot D.** The impact of embryonic development and endometrial maturity on the timing of implantation. *Fertil Steril*. 1992;58:537–542.
449. **Achache H, Revel A.** Endometrial receptivity markers, the journey to successful embryo implantation. *Hum Reprod Update*. 2006;12:731–746.
450. **Diaz S, Ortiz ME, Croxatto HB.** Studies on the duration of ovum transport by the human oviduct. III. Time interval between the luteinizing hormone peak and recovery of ova by transcervical flushing of the uterus in normal women. *Am J Obstet Gynecol*. 1980;137:116–121.
451. **Hertig AT, Rock J.** Two human ova of the pre-villous stage, having a development age of about eight and nine days respectively. *Contrib Embryol Carnegie Inst*. 1945; 33:169–186.
452. **Wilcox AJ, Baird DD, Weinberg CR.** Time of implantation of the conceptus and loss of pregnancy. *N Engl J Med*. 1999;340:1796–1799.
453. **Mahendru AA, Daemen A, Everett TR, Wilkinson IB, McEniery CM, Abdallah Y, Timmerman D, Bourne T, Lees CC.** Impact of ovulation and implantation timing on first-trimester crown-rump length and gestational age. *Ultrasound Obstet Gynecol*. 2012;40:630–635.
454. **Jukic AM, Baird DD, Weinberg CR, McConnaughey DR, Wilcox AJ.** Length of human pregnancy and contributors to its natural variation. *Hum Reprod*. 2013;28:2848–2855.
455. **Jaddoe VW, de Jonge LL, Hofman A, Franco OH, Steegers EA, Gaillard R.** First trimester fetal growth restriction and cardiovascular risk factors in school age children: population based cohort study. *BMJ*. 2014;348:g14.
456. **Coutifaris C, Myers ER, Guzick DS, Diamond MP, Carson SA, Legro RS, McGovern PG, Schlaff WD, Carr BR, Steinkampf MP, Silva S, Vogel DL, Leppert PC, Network NNCRM.** Histological dating of timed endometrial biopsy tissue is not related to fertility status. *Fertil Steril*. 2004; 82:1264–1272.
457. **Nikas G, Drakakis P, Loutradis D, Mara-Skoufari C, Koumantakis E, Michalas S, Psychoyos A.** Uterine pinopodes as markers of the 'nidation window' in cycling women receiving exogenous oestradiol and progesterone. *Hum Reprod*. 1995;10:1208–1213.
458. **Leach RE, Jessmon P, Coutifaris C, Kruger M, Myers ER, Ali-Fehmi R, Carson SA, Legro RS, Schlaff WD, Carr BR, Steinkampf MP, Silva S, Leppert PC, Giudice L, Diamond MP, Armant DR.** High throughput, cell type-specific anal-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

ysis of key proteins in human endometrial biopsies of women from fertile and infertile couples. *Hum Reprod*. 2012;27:814–828.

459. **Dominguez F, Garrido-Gomez T, Lopez JA, Camafeita E, Quinonero A, Pellicer A, Simon C.** Proteomic analysis of the human receptive versus non-receptive endometrium using differential in-gel electrophoresis and MALDI-MS unveils stathmin 1 and annexin A2 as differentially regulated. *Hum Reprod*. 2009;24:2607–2617.
460. **Brosens JJ, Hodgetts A, Feroze-Zaidi F, Sherwin JR, Fusi L, Salker MS, Higham J, Rose GL, Kajihara T, Young SL, Lessey BA, Henriet P, Langford PR, Fazleabas AT.** Proteomic analysis of endometrium from fertile and infertile patients suggests a role for apolipoprotein A-I in embryo implantation failure and endometriosis. *Mol Hum Reprod*. 2010;16:273–285.
461. **Boomsma CM, Kavelaars A, Eijkemans MJ, Lentjes EG, Fauser BC, Heijnen CJ, Macklon NS.** Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF. *Hum Reprod*. 2009;24:1427–1435.
462. **Boomsma CM, Kavelaars A, Eijkemans MJ, Amarouchi K, Teklenburg G, Gutknecht D, Fauser BJ, Heijnen CJ, Macklon NS.** Cytokine profiling in endometrial secretions: a non-invasive window on endometrial receptivity. *Reprod Biomed Online*. 2009;18:85–94.
463. **Casado-Vela J, Rodriguez-Suarez E, Iloro I, Ametzazurra A, Alkorta N, Garcia-Velasco JA, Matorras R, Prieto B, Gonzalez S, Nagore D, Simon L, Elortza F.** Comprehensive proteomic analysis of human endometrial fluid aspirate. *J Proteome Res*. 2009;8:4622–4632.
464. **Hannan NJ, Stephens AN, Rainczuk A, Hincks C, Rombauts LJ, Salamonsen LA.** 2D-DiGE analysis of the human endometrial secretome reveals differences between receptive and nonreceptive states in fertile and infertile women. *J Proteome Res*. 2010;9:6256–6264.
465. **Scotchie JG, Fritz MA, Mocanu M, Lessey BA, Young SL.** Proteomic analysis of the luteal endometrial secretome. *Reprod Sci*. 2009;16:883–893.
466. **Vilella F, Ramirez LB, Simon C.** Lipidomics as an emerging tool to predict endometrial receptivity. *Fertil Steril*. 2013; 99:1100–1106.
467. **Carson DD, Lagow E, Thathiah A, Al-Shami R, Farach-Carson MC, Vernon M, Yuan L, Fritz MA, Lessey B.** Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening. *Mol Hum Reprod*. 2002;8:871–879.
468. **Riesewijk A, Martin J, van Os R, Horcajadas JA, Polman J, Pellicer A, Mosselman S, Simon C.** Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology. *Mol Hum Reprod*. 2003;9:253–264.
469. **Talbi S, Hamilton AE, Vo KC, Tulac S, Overgaard MT, Dosiou C, Le Shay N, Nezhat CN, Kempson R, Lessey BA, Nayak NR, Giudice LC.** Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. *Endocrinology*. 2006;147:1097–1121.
470. **Haouzi D, Mahmoud K, Fourar M, Bendhaou K, Dechaud H, De Vos J, Reme T, Dewailly D, Hamamah S.** Identification of new biomarkers of human endometrial receptivity in the natural cycle. *Hum Reprod*. 2009;24:198–205.
471. **Diaz-Gimeno P, Horcajadas JA, Martinez-Conejero JA, Esteban FJ, Alama P, Pellicer A, Simon C.** A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. *Fertil Steril*. 2011;95:50–60.
472. **Ruiz-Alonso M, Blesa D, Diaz-Gimeno P, Gomez E, Fernandez-Sanchez M, Carranza F, Carrera J, Vilella F, Pellicer A, Simon C.** The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. *Fertil Steril*. 2013;100:818–824.
473. **Haouzi D, Dechaud H, Assou S, De Vos J, Hamamah S.** Insights into human endometrial receptivity from transcriptomic and proteomic data. *Reprod Biomed Online*. 2012;24:23–34.
474. **Edgell TA, Rombauts LJ, Salamonsen LA.** Assessing receptivity in the endometrium: the need for a rapid, non-invasive test. *Reprod Biomed Online*. 2013;27:486–496.
475. **Hoversland RC, Dey SK, Johnson DC.** Catechol estradiol induced implantation in the mouse. *Life Sci*. 1982;30: 1801–1804.
476. **Paria BC, Huet-Hudson YM, Dey SK.** Blastocyst's state of activity determines the "window" of implantation in the receptive mouse uterus. *Proc Natl Acad Sci USA*. 1993; 90:10159–10162.
477. **Ghosh D, De P, Sengupta J.** Luteal phase ovarian oestrogen is not essential for implantation and maintenance of pregnancy from surrogate embryo transfer in the rhesus monkey. *Hum Reprod*. 1994;9:629–637.
478. **Teklenburg G, Salker M, Heijnen C, Macklon NS, Brosens JJ.** The molecular basis of recurrent pregnancy loss: impaired natural embryo selection. *Mol Hum Reprod*. 2010; 16:886–895.
479. **Brosens JJ, Gellersen B.** Something new about early pregnancy: decidual biosensoring and natural embryo selection. *Ultrasound Obstet Gynecol*. 2010;36:1–5.
480. **Brosens JJ, Salker MS, Teklenburg G, Nautiyal J, Salter S, Lucas ES, Steel JH, Christian M, Chan YW, Boomsma CM, Moore JD, Hartshorne GM, Sucurovic S, Mulac-Jericevic B, Heijnen CJ, Quenby S, Groot Koerkamp MJ, Holstege FC, Shmygol A, Macklon NS.** Uterine selection of human embryos at implantation. *Sci Rep*. 2014;4:3894.
481. **Fragouli E, Alfarawati S, Spath K, Jaroudi S, Sarasa J, Enciso M, Wells D.** The origin and impact of embryonic aneuploidy. *Hum Genet*. 2013;132:1001–1013.
482. **Mertzanidou A, Wilton L, Cheng J, Spits C, Vanneste E, Moreau Y, Vermeesch JR, Sermon K.** Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos. *Hum Reprod*. 2013;28:256–264.
483. **Vanneste E, Voet T, Le Caignec C, Ampe M, Konings P, Melotte C, Debrock S, Amyere M, Vikkula M, Schuit F, Fryns JP, Verbeke G, D'Hooghe T, Moreau Y, Vermeesch JR.** Chromosome instability is common in human cleavage-stage embryos. *Nat Med*. 2009;15:577–583.
484. **Gräfenberg E.** Beiträge zur Physiologie der Eieinbettung. *Z Geburtsh Gynäkol*. 1910;65:1–35.
485. **Noyes RW.** Trophoblast: problems of invasion and transport. *Ann N Y Acad Sci*. 1959;80:54–64.
486. **Pijnenborg R, Robertson WB, Brosens I, Dixon G.** Review

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

article: trophoblast invasion and the establishment of haemochorial placentation in man and laboratory animals. *Placenta*. 1981;2:71–91.

487. **Anacker J, Segerer SE, Hagemann C, Feix S, Kapp M, Bausch R, Kämmerer U.** Human decidua and invasive trophoblasts are rich sources of nearly all human matrix metalloproteinases. *Mol Hum Reprod*. 2011;17:637–652.
488. **Weimar CHE, Macklon NS, Post Uiterweer ED, Brosens JJ, Gellersen B.** The motile and invasive capacity of human endometrial stromal cells: implications for normal and impaired reproductive function. *Hum Reprod Update*. 2013; 19:542–557.
489. **Grewal S, Carver JG, Ridley AJ, Mardon HJ.** Implantation of the human embryo requires Rac1-dependent endometrial stromal cell migration. *Proc Natl Acad Sci USA*. 2008; 105:16189–16194.
490. **Grewal S, Carver J, Ridley AJ, Mardon HJ.** Human endometrial stromal cell Rho GTPases have opposing roles in regulating focal adhesion turnover and embryo invasion in vitro. *Biol Reprod*. 2010;83:75–82.
491. **Spessotto P, Bulla R, Danussi C, Radillo O, Cervi M, Monami G, Bossi F, Tedesco F, Doliana R, Colombatti A.** EMILIN1 represents a major stromal element determining human trophoblast invasion of the uterine wall. *J Cell Sci*. 2006;119:4574–4584.
492. **Gellersen B, Reimann K, Samalecos A, Aupers S, Bamberger AM.** Invasiveness of human endometrial stromal cells is promoted by decidualization and by trophoblast-derived signals. *Hum Reprod*. 2010;25:862–873.
493. **Ren L, Liu YQ, Zhou WH, Zhang YZ.** Trophoblast-derived chemokine CXCL12 promotes CXCR4 expression and invasion of human first-trimester decidual stromal cells. *Hum Reprod*. 2012;27:366–374.
494. **Weimar CHE, Kavelaars A, Brosens JJ, Gellersen B, de Vreeden-Elbertse JMT, Heijnen CJ, Macklon NS.** Endometrial stromal cells of women with recurrent miscarriage fail to discriminate between high- and low-quality human embryos. *PLoS ONE*. 2012;7:e41424.
495. **Haouzi D, Dechaud H, Assou S, Monzo C, de Vos J, Hamamah S.** Transcriptome analysis reveals dialogues between human trophectoderm and endometrial cells during the implantation period. *Hum Reprod*. 2011;26:1440–1449.
496. **Babawale MO, Mobberley MA, Ryder TA, Elder MG, Sullivan MH.** Ultrastructure of the early human feto-maternal interface co-cultured *in vitro*. *Hum Reprod*. 2002; 17:1351–1357.
497. **Quenby S, Brosens JJ.** Human implantation: a tale of mutual maternal and fetal attraction. *Biol Reprod 88*:. 2013; 81:81–82.
498. **Mansouri-Attia N, Sandra O, Aubert J, Degrelle S, Everts RE, Giraud-Delville C, Heyman Y, Galio L, Hue I, Yang X, Tian XC, Lewin HA, Renard JP.** Endometrium as an early sensor of in vitro embryo manipulation technologies. *Proc Natl Acad Sci USA*. 2009;106:5687–5692.
499. **Barnes FL.** The effects of the early uterine environment on the subsequent development of embryo and fetus. *Theriogenol*. 2000;53:649–658.
500. **Kampinga HH, Craig EA.** The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat Rev Mol Cell Biol*. 2010;11:579–592.
501. **Ruan YC, Guo JH, Liu X, Zhang R, Tsang LL, Dong JD, Chen H, Yu MK, Jiang X, Zhang XH, Fok KL, Chung YW, Huang H, Zhou WL, Chan HC.** Activation of the epithelial Na^+ channel triggers prostaglandin E_2 release and production required for embryo implantation. *Nat Med*. 2012;18:1112–1117.
502. **Medawar PB.** Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. *Symp Soc Exp Biol*. 1953;7:320–338.
503. **Moffett A, Loke C.** Immunology of placentation in eutherian mammals. *Nat Rev Immunol*. 2006;6:584–594.
504. **Erlebacher A.** Immunology of the maternal-fetal interface. *Annu Rev Immunol*. 2013;31:387–411.
505. **Erlebacher A.** Mechanisms of T cell tolerance towards the allogeneic fetus. *Nat Rev Immunol*. 2013;13:23–33.
506. **Veenstra van Nieuwenhoven AL, Heineman MJ, Faas MM.** The immunology of successful pregnancy. *Hum Reprod Update*. 2003;9:347–357.
507. **Kämmerer U, Schoppet M, McLellan AD, Kapp M, Huppertz HI, Kampgen E, Dietl J.** Human decidua contains potent immunostimulatory $CD83^+$ dendritic cells. *Am J Pathol*. 2000;157:159–169.
508. **Kemp B, Schmitz S, Krusche CA, Rath W, von Rango U.** Dendritic cells are equally distributed in intrauterine and tubal ectopic pregnancies. *Fertil Steril*. 2011;95:28–32.
509. **Collins MK, Tay CS, Erlebacher A.** Dendritic cell entrapment within the pregnant uterus inhibits immune surveillance of the maternal/fetal interface in mice. *J Clin Invest*. 2009;119:2062–2073.
510. **Tagliani E, Erlebacher A.** Dendritic cell function at the maternal-fetal interface. *Expert Rev Clin Immunol*. 2011; 7:593–602.
511. **Volchek M, Girling JE, Lash GE, Cann L, Kumar B, Robson SC, Bulmer JN, Rogers PA.** Lymphatics in the human endometrium disappear during decidualization. *Hum Reprod*. 2010;25:2455–2464.
512. **Red-Horse K.** Lymphatic vessel dynamics in the uterine wall. *Placenta* 2008;29 Suppl A:S55–59.
513. **Red-Horse K, Rivera J, Schanz A, Zhou Y, Winn V, Kapidzic M, Maltepe E, Okazaki K, Kochman R, Vo KC, Giudice L, Erlebacher A, McCune JM, Stoddart CA, Fisher SJ.** Cytotrophoblast induction of arterial apoptosis and lymphangiogenesis in an in vivo model of human placentation. *J Clin Invest*. 2006;116:2643–2652.
514. **Nancy P, Tagliani E, Tay CS, Asp P, Levy DE, Erlebacher A.** Chemokine gene silencing in decidual stromal cells limits T cell access to the maternal-fetal interface. *Science*. 2012;336:1317–1321.
515. **Bevan BH, Kilpatrick DC, Liston WA, Hirabayashi J, Kasai K.** Immunohistochemical localization of a beta-D-galactoside-binding lectin at the human maternofetal interface. *Histochem J*. 1994;26:582–586.
516. **von Wolff M, Wang X, Gabius HJ, Strowitzki T.** Galectin fingerprinting in human endometrium and decidua during the menstrual cycle and in early gestation. *Mol Hum Reprod*. 2005;11:189–194.
517. **Barrientos G, Freitag N, Tirado-Gonzalez I, Unverdorben L, Jeschke U, Thijssen VL, Blois SM.** Involvement of galectin-1 in reproduction: past, present and future. *Hum Reprod Update*. 2014;20:175–193.
518. **Camby I, Le Mercier M, Lefranc F, Kiss R.** Galectin-1: a

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

small protein with major functions. *Glycobiology*. 2006; 16:137R–157R.
519. **Carlsson MC, Cederfur C, Schaar V, Balog CI, Lepur A, Touret F, Salomonsson E, Deelder AM, Ferno M, Olsson H, Wuhrer M, Leffler H.** Galectin-1-binding glycoforms of haptoglobin with altered intracellular trafficking, and increase in metastatic breast cancer patients. *PLoS ONE*. 2011;6:e26560.
520. **Blois SM, Ilarregui JM, Tometten M, Garcia M, Orsal AS, Cordo-Russo R, Toscano MA, Bianco GA, Kobelt P, Handjiski B, Tirado I, Markert UR, Klapp BF, Poirier F, Szekeres-Bartho J, Rabinovich GA, Arck PC.** A pivotal role for galectin-1 in fetomaternal tolerance. *Nat Med*. 2007; 13:1450–1457.
521. **Kopcow HD, Rosetti F, Leung Y, Allan DS, Kutok JL, Strominger JL.** T cell apoptosis at the maternal-fetal interface in early human pregnancy, involvement of galectin-1. *Proc Natl Acad Sci USA*. 2008;105:18472–18477.
522. **Mellor AL, Chandler P, Lee GK, Johnson T, Keskin DB, Lee J, Munn DH.** Indoleamine 2,3-dioxygenase, immunosuppression and pregnancy. *J Reprod Immunol*. 2002;57: 143–150.
523. **Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL.** Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science*. 1998; 281:1191–1193.
524. **Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, Prus D, Cohen-Daniel L, Arnon TI, Manaster I, Gazit R, Yutkin V, Benharroch D, Porgador A, Keshet E, Yagel S, Mandelboim O.** Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med*. 2006;12:1065–1074.
525. **Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, Schatz F, Masch R, Lockwood CJ, Schachter AD, Park PJ, Strominger JL.** Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *J Exp Med*. 2003;198:1201–1212.
526. **Moffett-King A.** Natural killer cells and pregnancy. *Nat Rev Immunol*. 2002;2:656–663.
527. **Lash GE, Schiessl B, Kirkley M, Innes BA, Cooper A, Searle RF, Robson SC, Bulmer JN.** Expression of angiogenic growth factors by uterine natural killer cells during early pregnancy. *J Leukoc Biol*. 2006;80:572–580.
528. **Keskin DB, Allan DS, Rybalov B, Andzelm MM, Stern JN, Kopcow HD, Koopman LA, Strominger JL.** TGFβ promotes conversion of $CD16^+$ peripheral blood NK cells into $CD16^-$ NK cells with similarities to decidual NK cells. *Proc Natl Acad Sci USA*. 2007;104:3378–3383.
529. **Vacca P, Vitale C, Montaldo E, Conte R, Cantoni C, Fulcheri E, Darretta V, Moretta L, Mingari MC.** $CD34^+$ hematopoietic precursors are present in human decidua and differentiate into natural killer cells upon interaction with stromal cells. *Proc Natl Acad Sci USA*. 2011;108: 2402–2407.
530. **Cerdeira AS, Rajakumar A, Royle CM, Lo A, Husain Z, Thadhani RI, Sukhatme VP, Karumanchi SA, Kopcow HD.** Conversion of peripheral blood NK cells to a decidual NK-like phenotype by a cocktail of defined factors. *J Immunol*. 2013;190:3939–3948.
531. **Wallace AE, Fraser R, Cartwright JE.** Extravillous trophoblast and decidual natural killer cells: a remodelling partnership. *Hum Reprod Update*. 2012;18:458–471.
532. **Fraser R, Whitley GS, Johnstone AP, Host AJ, Sebire NJ, Thilaganathan B, Cartwright JE.** Impaired decidual natural killer cell regulation of vascular remodelling in early human pregnancies with high uterine artery resistance. *J Pathol*. 2012;228:322–332.
533. **Zhang J, Chen Z, Smith GN, Croy BA.** Natural killer cell-triggered vascular transformation: maternal care before birth? *Cell Mol Immunol*. 2011;8:1–11.
534. **Hiby SE, Apps R, Sharkey AM, Farrell LE, Gardner L, Mulder A, Claas FH, Walker JJ, Redman CW, Morgan L, Tower C, Regan L, Moore GE, Carrington M, Moffett A.** Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2. *J Clin Invest*. 2010;120:4102–4110.
535. **Kieckbusch J, Gaynor LM, Moffett A, Colucci F.** MHC-dependent inhibition of uterine NK cells impedes fetal growth and decidual vascular remodelling. *Nat Commun*. 2014;5:3359.
536. **Helige C, Ahammer H, Moser G, Hammer A, Dohr G, Huppertz B, Sedlmayr P.** Distribution of decidual natural killer cells and macrophages in the neighbourhood of the trophoblast invasion front: a quantitative evaluation. *Hum Reprod*. 2014;29:8–17.
537. **Renaud SJ, Graham CH.** The role of macrophages in uteroplacental interactions during normal and pathological pregnancy. *Immunol Invest*. 2008;37:535–564.
538. **Bulmer JN, Williams PJ, Lash GE.** Immune cells in the placental bed. *Int J Dev Biol*. 2010;54:281–294.
539. **Cupurdija K, Azzola D, Hainz U, Gratchev A, Heitger A, Takikawa O, Goerdt S, Wintersteiger R, Dohr G, Sedlmayr P.** Macrophages of human first trimester decidua express markers associated to alternative activation. *Am J Reprod Immunol*. 2004;51:117–122.
540. **Svensson J, Jenmalm MC, Matussek A, Geffers R, Berg G, Ernerudh J.** Macrophages at the fetal-maternal interface express markers of alternative activation and are induced by M-CSF and IL-10. *J Immunol*. 2011;187:3671–3682.
541. **Mor G, Abrahams VM.** Potential role of macrophages as immunoregulators of pregnancy. *Reprod Biol Endocrinol*. 2003;1:119.
542. **Wynn TA, Chawla A, Pollard JW.** Macrophage biology in development, homeostasis and disease. *Nature*. 2013;496: 445–455.
543. **Houser BL, Tilburgs T, Hill J, Nicotra ML, Strominger JL.** Two unique human decidual macrophage populations. *J Immunol*. 2011;186:2633–2642.
544. **Houser BL.** Decidual macrophages and their roles at the maternal-fetal interface. *The Yale journal of biology and medicine*. 2012;85:105–118.
545. **Co EC, Gormley M, Kapidzic M, Rosen DB, Scott MA, Stolp HA, McMaster M, Lanier LL, Barcena A, Fisher SJ.** Maternal decidual macrophages inhibit NK cell killing of invasive cytotrophoblasts during human pregnancy. *Biol Reprod*. 2013;88:155.
546. **Vacca P, Cantoni C, Vitale M, Prato C, Canegallo F, Fenoglio D, Ragni N, Moretta L, Mingari MC.** Crosstalk between decidual NK and $CD14^+$ myelomonocytic cells results in induction of Tregs and immunosuppression. *Proc Natl Acad Sci USA*. 2010;107:11918–11923.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

547. **Croxatto D, Vacca P, Canegallo F, Conte R, Venturini PL, Moretta L, Mingari MC.** Stromal cells from human decidua exert a strong inhibitory effect on NK cell function and dendritic cell differentiation. *PLoS ONE*. 2014;9: e89006.
548. **Erkers T, Nava S, Yosef J, Ringden O, Kaipe H.** Decidual stromal cells promote regulatory T cells and suppress alloreactivity in a cell contact-dependent manner. *Stem Cells Dev*. 2013;22:2596–2605.
549. **Ringdén O, Erkers T, Nava S, Uzunel M, Iwarsson E, Conrad R, Westgren M, Mattsson J, Kaipe H.** Fetal membrane cells for treatment of steroid-refractory acute graft-versus-host disease. *Stem Cells*. 2013;31:592–601.
550. **Leitao BB, Jones MC, Brosens JJ.** The SUMO E3-ligase PIAS1 couples reactive oxygen species-dependent JNK activation to oxidative cell death. *FASEB J*. 2011;25:3416–3425.
551. **Macklon NS, Geraedts JP, Fauser BC.** Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. *Hum Reprod Update*. 2002;8:333–343.
552. **Promislow JH, Baird DD, Wilcox AJ, Weinberg CR.** Bleeding following pregnancy loss before 6 weeks' gestation. *Hum Reprod*. 2007;22:853–857.
553. **Talmadge K, Boorstein WR, Fiddes JC.** The human genome contains seven genes for the beta-subunit of chorionic gonadotropin but only one gene for the beta-subunit of luteinizing hormone. *DNA*. 1983;2:281–289.
554. **Hallast P, Nagirnaja L, Margus T, Laan M.** Segmental duplications and gene conversion: Human luteinizing hormone/chorionic gonadotropin beta gene cluster. *Genome Res*. 2005;15:1535–1546.
555. **Brosens JJ, Salker MS, Teklenburg G, Nautiyal J, Salter S, Lucas ES, Steel JH, Christian M, Chan YW, Boomsma CM, Moore JD, Hartshorne GM, Sucurovic S, Mulac-Jericevic B, Heijnen CJ, Quenby S, Koerkamp MJ, Holstege FC, Shmygol A, Macklon NS.** Uterine selection of human embryos at implantation. *Sci Rep*. 2014;4:3894.
556. **Jabbour HN, Kelly RW, Fraser HM, Critchley HO.** Endocrine regulation of menstruation. *Endocr Rev*. 2006;27: 17–46.
557. **Evans J, Salamonsen LA.** Inflammation, leukocytes and menstruation. *Rev Endocr Metab Disord*. 2012;13:277–288.
558. **Henriet P, Gaide Chevronnay HP, Marbaix E.** The endocrine and paracrine control of menstruation. *Mol Cell Endocrinol*. 2012;358:197–207.
559. **Markee JE.** Menstruation in intraocular endometrial transplants in the rhesus monkey. *Contrib Embryol Carnegie Inst*. 1940;28:219–308.
560. **Sharkey AM, Day K, McPherson A, Malik S, Licence D, Smith SK, Charnock-Jones DS.** Vascular endothelial growth factor expression in human endometrium is regulated by hypoxia. *J Clin Endocrinol Metab*. 2000;85:402–409.
561. **Coudyzer P, Lemoine P, Jordan BF, Gallez B, Galant C, Nisolle M, Courtoy PJ, Henriet P, Marbaix E.** Hypoxia is not required for human endometrial breakdown or repair in a xenograft model of menstruation. *FASEB J*. 2013;27: 3711–3719.
562. **Brenner RM, Nayak NR, Slayden OD, Critchley HO, Kelly RW.** Premenstrual and menstrual changes in the macaque and human endometrium: relevance to endometriosis. *Ann N Y Acad Sci 955:60–74; discussion 86-*. 2002; 68:396–406.
563. **Slayden OD, Brenner RM.** A critical period of progesterone withdrawal precedes menstruation in macaques. *Reprod Biol Endocrinol 4 Suppl*. 2006;1:S6.
564. **Evans J, Salamonsen LA.** Decidualized human endometrial stromal cells are sensors of hormone withdrawal in the menstrual inflammatory cascade. *Biol Reprod*. 2014;90: 14.
565. **Sugino N, Karube-Harada A, Taketani T, Sakata A, Nakamura Y.** Withdrawal of ovarian steroids stimulates prostaglandin F2α production through nuclear factor κB activation via oxygen radicals in human endometrial stromal cells: potential relevance to menstruation. *J Reprod Dev*. 2004;50:215–225.
566. **Cha J, Bartos A, Egashira M, Haraguchi H, Saito-Fujita T, Leishman E, Bradshaw H, Dey SK, Hirota Y.** Combinatory approaches prevent preterm birth profoundly exacerbated by gene-environment interactions. *J Clin Invest*. 2013;123: 4063–4075.
567. **Finn CA.** Menstruation: a nonadaptive consequence of uterine evolution. *Q Rev Biol*. 1998;73:163–173.
568. **Finn CA.** Why do women menstruate? Historical and evolutionary review. *Eur J Obstet Gynecol Reprod Biol*. 1996; 70:3–8.
569. **Taylor HS.** Endometrial cells derived from donor stem cells in bone marrow transplant recipients. *J Am Med Assoc*. 2004;292:81–85.
570. **Mints M, Jansson M, Sadeghi B, Westgren M, Uzunel M, Hassan M, Palmblad J.** Endometrial endothelial cells are derived from donor stem cells in a bone marrow transplant recipient. *Hum Reprod*. 2008;23:139–143.
571. **Ikoma T, Kyo S, Maida Y, Ozaki S, Takakura M, Nakao S, Inoue M.** Bone marrow-derived cells from male donors can compose endometrial glands in female transplant recipients. *Am J Obstet Gynecol* 2009;201:608 e601–608.
572. **Du H, Taylor HS.** Contribution of bone marrow-derived stem cells to endometrium and endometriosis. *Stem Cells*. 2007;25:2082–2086.
573. **Bratincsak A, Brownstein MJ, Cassiani-Ingoni R, Pastorino S, Szalayova I, Toth ZE, Key S, Nemeth K, Pickel J, Mezey E.** CD45-positive blood cells give rise to uterine epithelial cells in mice. *Stem Cells*. 2007;25:2820–2826.
574. **Du H, Naqvi H, Taylor HS.** Ischemia/reperfusion injury promotes and granulocyte-colony stimulating factor inhibits migration of bone marrow-derived stem cells to endometrium. *Stem Cells Dev*. 2012;21:3324–3331.
575. **Wheeler MD.** Physical changes of puberty. *Endocrinol Metab Clin North Am*. 1991;20:1–14.
576. **Brosens JJ, Parker MG, McIndoe A, Pijnenborg R, Brosens IA.** A role for menstruation in preconditioning the uterus for successful pregnancy. *Am J Obstet Gynecol* 2009;200: 615 e611–616.
577. **Garry R, Hart R, Karthigasu KA, Burke C.** A re-appraisal of the morphological changes within the endometrium during menstruation: a hysteroscopic, histological and scanning electron microscopic study. *Hum Reprod*. 2009; 24:1393–1401.
578. **Ludwig H, Spornitz UM.** Microarchitecture of the human endometrium by scanning electron microscopy: menstrual

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

desquamation and remodeling. *Ann N Y Acad Sci*. 1991; 622:28–46.
579. **Ferenczy A.** Studies on the cytodynamics of human endometrial regeneration. I. Scanning electron microscopy. *Am J Obstet Gynecol*. 1976;124:64–74.
580. **Cousins FL, Murray A, Esnal A, Gibson DA, Critchley HO, Saunders PT.** Evidence from a mouse model that epithelial cell migration and mesenchymal-epithelial transition contribute to rapid restoration of uterine tissue integrity during menstruation. *PLoS ONE*. 2014;9:e86378.
581. **Franasiak JM, Holoch KJ, Yuan L, Schammel DP, Young SL, Lessey BA.** Prospective assessment of midsecretory endometrial leukemia inhibitor factor expression versus $\alpha v\beta 3$ testing in women with unexplained infertility. *Fertil Steril*. 2014;101:1724–1731.
582. **Eskenazi B, Warner ML.** Epidemiology of endometriosis. *Obstet Gynecol Clin North Am*. 1997;24:235–258.
583. **Nnoaham KE, Webster P, Kumbang J, Kennedy SH, Zondervan KT.** Is early age at menarche a risk factor for endometriosis? A systematic review and meta-analysis of case-control studies. *Fertil Steril*. 2012;98:702–712 e706.
584. **Sampson J.** Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol*. 1927;14:422–429.
585. **Klemmt PA, Carver JG, Kennedy SH, Koninckx PR, Mardon HJ.** Stromal cells from endometriotic lesions and endometrium from women with endometriosis have reduced decidualization capacity. *Fertil Steril*. 2006;85:564–572.
586. **Aghajanova L, Hamilton A, Kwintkiewicz J, Vo KC, Giudice LC.** Steroidogenic enzyme and key decidualization marker dysregulation in endometrial stromal cells from women with versus without endometriosis. *Biol Reprod*. 2009;80:105–114.
587. **Minici F, Tiberi F, Tropea A, Orlando M, Gangale MF, Romani F, Campo S, Bompiani A, Lanzone A, Apa R.** Endometriosis and human infertility: a new investigation into the role of eutopic endometrium. *Hum Reprod*. 2008; 23:530–537.
588. **Yin X, Pavone ME, Lu Z, Wei J, Kim JJ.** Increased activation of the PI3K/AKT pathway compromises decidualization of stromal cells from endometriosis. *J Clin Endocrinol Metab*. 2012;97:E35–43.
589. **Yang H, Zhou Y, Edelshain B, Schatz F, Lockwood CJ, Taylor HS.** FKBP4 is regulated by HOXA10 during decidualization and in endometriosis. *Reproduction*. 2012; 143:531–538.
590. **Brosens I, Brosens JJ, Benagiano G.** The eutopic endometrium in endometriosis: are the changes of clinical significance? *Reprod Biomed Online*. 2012;24:496–502.
591. **Benagiano G, Brosens I, Habiba M.** Structural and molecular features of the endomyometrium in endometriosis and adenomyosis. *Hum Reprod Update*. 2014;20:386–402.
592. **Al-Sabbagh M, Lam EW, Brosens JJ.** Mechanisms of endometrial progesterone resistance. *Mol Cell Endocrinol*. 2012;358:208–215.
593. **Lessey BA, Lebovic DI, Taylor RN.** Eutopic endometrium in women with endometriosis: ground zero for the study of implantation defects. *Semin Reprod Med*. 2013;31:109–124.
594. **Han SJ, Hawkins SM, Begum K, Jung SY, Kovanci E, Qin J, Lydon JP, DeMayo FJ, O'Malley BW.** A new isoform of steroid receptor coactivator-1 is crucial for pathogenic progression of endometriosis. *Nat Med*. 2012;18:1102–1111.
595. **Fazleabas AT.** Progesterone resistance in a baboon model of endometriosis. *Semin Reprod Med*. 2010;28:75–80.
596. **Grümmer R.** Translational animal models to study endometriosis-associated infertility. *Semin Reprod Med*. 2013; 31:125–132.
597. **Bulun SE, Cheng YH, Yin P, Imir G, Utsunomiya H, Attar E, Innes J, Julie Kim J.** Progesterone resistance in endometriosis: link to failure to metabolize estradiol. *Mol Cell Endocrinol*. 2006;248:94–103.
598. **Velarde MC, Aghajanova L, Nezhat CR, Giudice LC.** Increased mitogen-activated protein kinase kinase/extracellularly regulated kinase activity in human endometrial stromal fibroblasts of women with endometriosis reduces 3′,5′-cyclic adenosine 5′-monophosphate inhibition of cyclin D1. *Endocrinology*. 2009;150:4701–4712.
599. **Sherwin JR, Hastings JM, Jackson KS, Mavrogianis PA, Sharkey AM, Fazleabas AT.** The endometrial response to chorionic gonadotropin is blunted in a baboon model of endometriosis. *Endocrinology*. 2010;151:4982–4993.
600. **Heitmann RJ, Langan KL, Huang RR, Chow GE, Burney RO.** Premenstrual spotting of two or more days is strongly associated with histologically confirmed endometriosis in women with infertility. *Am J Obstet Gynecol*. 2014; [Epub ahead of print] DOI: 10.1016/j.ajog. 2014.04.041.
601. **Stephansson O, Kieler H, Granath F, Falconer H.** Endometriosis, assisted reproduction technology, and risk of adverse pregnancy outcome. *Hum Reprod*. 2009;24: 2341–2347.
602. **Fernando S, Breheny S, Jaques AM, Halliday JL, Baker G, Healy D.** Preterm birth, ovarian endometriomata, and assisted reproduction technologies. *Fertil Steril*. 2009;91: 325–330.
603. **Brosens I, Brosens JJ, Fusi L, Al-Sabbagh M, Kuroda K, Benagiano G.** Risks of adverse pregnancy outcome in endometriosis. *Fertil Steril*. 2012;98:30–35.
604. **Naqvi H, Sakr S, Presti T, Krikun G, Komm B, Taylor HS.** Treatment with Bazedoxifene and conjugated estrogens results in regression of endometriosis in a murine model. *Biol Reprod*. 2014;90:121.
605. **Gargett CE, Schwab KE, Brosens JJ, Puttemans P, Benagiano G, Brosens I.** Potential role of endometrial stem/progenitor cells in the pathogenesis of early-onset endometriosis. *Mol Hum Reprod*. 2014;20:591–598.
606. **Kaiser R, Grassel G.** [Incidence and intensity of uterine bleeding in the neonate (author's transl)]. *Geburtshilfe Frauenheilkd*. 1974;34:644–648.
607. **Ober WB, Bernstein J.** Observations on the endometrium and ovary in the newborn. *Pediatrics*. 1955;16:445–460.
608. **Health and Social Care Information Center.** 2013 Hospital episode statistics, NHS maternity statistics 2012–2013, London HSCIC. Available at: http://www.hscic.gov.uk/catalogue/PUB12744.
609. **Rai R, Regan L.** Recurrent miscarriage. *Lancet*. 2006;368: 601–611.
610. **Bhattacharya S, Bhattacharya S.** Effect of miscarriage on future pregnancies. *Women's health*. 2009;5:5–8.
611. **Jauniaux E, Farquharson RG, Christiansen OB, Exalto N.** Evidence-based guidelines for the investigation and med-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

ical treatment of recurrent miscarriage. *Hum Reprod*. 2006;21:2216–2222.
612. **Vlachadis N, Tsamadias V, Economou E.** Association between miscarriage and future maternal cardiovascular disease. *Heart*. 2013;99:1706.
613. **Practice Committee of the American Society for Reproductive Medicine.** Definitions of infertility and recurrent pregnancy loss. *Fertil Steril*. 2008;89:1603.
614. **Farquharson RG, Jauniaux E, Exalto N, ESHRE Special Interest Group for Early Pregnancy.** Updated and revised nomenclature for description of early pregnancy events. *Hum Reprod*. 2005;20:3008–3011.
615. **Rodger MA, Betancourt MT, Clark P, Lindqvist PG, Dizon-Townson D, Said J, Seligsohn U, Carrier M, Salomon O, Greer IA.** The association of factor V leiden and prothrombin gene mutation and placenta-mediated pregnancy complications: a systematic review and meta-analysis of prospective cohort studies. *PLoS Med*. 2010;7:e1000292.
616. **Haas DM, Ramsey PS.** Progestogen for preventing miscarriage. *Cochrane Database Syst Rev*. 2013;10:CD003511.
617. **Haraldsen G, Balogh J, Pollheimer J, Sponheim J, Kuchler AM.** Interleukin-33 - cytokine of dual function or novel alarmin? *Trends Immunol*. 2009;30:227–233.
618. **Salker MS, Christian M, Steel JH, Nautiyal J, Lavery S, Trew G, Webster Z, Al-Sabbagh M, Puchchakayala G, Foller M, Landles C, Sharkey AM, Quenby S, Aplin JD, Regan L, Lang F, Brosens JJ.** Deregulation of the serum- and glucocorticoid-inducible kinase SGK1 in the endometrium causes reproductive failure. *Nat Med*. 2011;17:1509–1513.
619. **van Deursen JM.** The role of senescent cells in ageing. *Nature*. 2014;509:439–446.
620. **Krizhanovsky V, Yon M, Dickins RA, Hearn S, Simon J, Miething C, Yee H, Zender L, Lowe SW.** Senescence of activated stellate cells limits liver fibrosis. *Cell*. 2008;134:657–667.
621. **Kaandorp SP, Goddijn M, van der Post JA, Hutten BA, Verhoeve HR, Hamulyak K, Mol BW, Folkeringa N, Nahuis M, Papatsonis DN, Buller HR, van der Veen F, Middeldorp S.** Aspirin plus heparin or aspirin alone in women with recurrent miscarriage. *N Engl J Med*. 2010;362:1586–1596.
622. **Clark P, Walker ID, Langhorne P, Crichton L, Thomson A, Greaves M, Whyte S, Greer IA, Scottish Pregnancy Intervention Study c.** SPIN (Scottish Pregnancy Intervention) study: a multicenter, randomized controlled trial of low-molecular-weight heparin and low-dose aspirin in women with recurrent miscarriage. *Blood* 2010;115:4162–4167.
623. **Wang X, Chen C, Wang L, Chen D, Guang W, French J.** Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study. *Fertil Steril*. 2003;79:577–584.
624. **Jauniaux E, Watson AL, Hempstock J, Bao YP, Skepper JN, Burton GJ.** Onset of maternal arterial blood flow and placental oxidative stress. A possible factor in human early pregnancy failure. *Am J Pathol*. 2000;157:2111–2122.
625. **Hempstock J, Jauniaux E, Greenwold N, Burton GJ.** The contribution of placental oxidative stress to early pregnancy failure. *Hum Pathol*. 2003;34:1265–1275.
626. **Einspanier A, Lieder K, Husen B, Ebert K, Lier S, Einspanier R, Unemori E, Kemper M.** Relaxin supports implantation and early pregnancy in the marmoset monkey. *Ann N Y Acad Sci*. 2009;1160:140–146.
627. **Barash A, Dekel N, Fieldust S, Segal I, Schechtman E, Granot I.** Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing in vitro fertilization. *Fertil Steril*. 2003;79:1317–1322.
628. **Potdar N, Gelbaya T, Nardo LG.** Endometrial injury to overcome recurrent embryo implantation failure: a systematic review and meta-analysis. *Reprod Biomed Online*. 2012;25:561–571.
629. **Samalecos A, Reimann K, Wittmann S, Schulte HM, Brosens JJ, Bamberger AM, Gellersen B.** Characterization of a novel telomerase-immortalized human endometrial stromal cell line, *St-T1b*. *Reprod Biol Endocrinol*. 2009;7:76.
630. **Ferrari A, Petraglia F, Gurpide E.** Corticotropin releasing factor decidualizes human endometrial stromal cells *in vitro*. *Interaction with progestin*. *J Steroid Biochem Mol Biol*. 1995;54:251–255.
631. **Tang B, Guller S, Gurpide E.** Cyclic adenosine 3′,5′-monophosphate induces prolactin expression in stromal cells isolated from human proliferative endometrium. *Endocrinology*. 1993;133:2197–2203.
632. **Tseng L, Gao JG, Chen R, Zhu HH, Mazella J, Powell DR.** Effect of progestin, antiprogestin, and relaxin on the accumulation of prolactin and insulin-like growth factor-binding protein-1 messenger ribonucleic acid in human endometrial stromal cells. *Biol Reprod*. 1992;47:441–450.
633. **Frank GR, Brar AK, Cedars MI, Handwerger S.** Prostaglandin E_2 enhances human endometrial stromal cell differentiation. *Endocrinology*. 1994;134:258–263.
634. **Rosenberg M, Mazella J, Tseng L.** Relative potency of relaxin, insulin-like growth factors, and insulin on the prolactin production in progestin-primed human endometrial stromal cells in long-term culture. *Ann N Y Acad Sci*. 1991;622:138–144.
635. **Huang JR, Tseng L, Bischof P, Jänne OA.** Regulation of prolactin production by progestin, estrogen, and relaxin in human endometrial stromal cells. *Endocrinology*. 1987;121:2011–2017.
636. **Zhu HH, Huang JR, Mazella J, Rosenberg M, Tseng L.** Differential effects of progestin and relaxin on the synthesis and secretion of immunoreactive prolactin in long term culture of human endometrial stromal cells. *J Clin Endocrinol Metab*. 1990;71:889–899.
637. **Irwin JC, Utian WH, Eckert RL.** Sex steroids and growth factors differentially regulate the growth and differentation of cultured human endometrial stromal cells. *Endocrinology*. 1991;129:2385–2392.
638. **Irwin JC, Kirk D, King RJB, Quigley MM, Gwatkin RBL.** Hormonal regulation of human endometrial stromal cells in culture: an in vitro model for decidualization. *Fertil Steril*. 1989;52:761–768.
639. **Dimitriadis E, Robb L, Salamonsen LA.** Interleukin 11 advances progesterone-induced decidualization of human endometrial stromal cells. *Mol Hum Reprod*. 2002;8:636–643.
640. **Hamaguchi M, Yamamoto T, Sugiyama Y.** Production of prolactin by cultures of isolated cells from human first-trimester decidua. *Obstet Gynecol*. 1990;76:783–787.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.

641. **Nagashima T, Li Q, Clementi C, Lydon JP, DeMayo FJ, Matzuk MM.** BMPR2 is required for postimplantation uterine function and pregnancy maintenance. *J Clin Invest*. 2013;123:2539–2550.
642. **Igakura T, Kadomatsu K, Kaname T, Muramatsu H, Fan QW, Miyauchi T, Toyama Y, Kuno N, Yuasa S, Takahashi M, Senda T, Taguchi O, Yamamura K, Arimura K, Muramatsu T.** A null mutation in basigin, an immunoglobulin superfamily member, indicates its important roles in peri-implantation development and spermatogenesis. *Dev Biol*. 1998;194:152–165.
643. **Reardon SN, King ML, MacLean JA, 2nd, Mann JL, DeMayo FJ, Lydon JP, Hayashi K.** CDH1 is essential for endometrial differentiation, gland development, and adult function in the mouse uterus. *Biol Reprod 86:*. 2012;141: 141–110.
644. **Wang W, Li Q, Bagchi IC, Bagchi MK.** The CCAAT/enhancer binding protein β is a critical regulator of steroid-induced mitotic expansion of uterine stromal cells during decidualization. *Endocrinology*. 2010;151:3929–3940.
645. **Jeong JW, Lee HS, Franco HL, Broaddus RR, Taketo MM, Tsai SY, Lydon JP, DeMayo FJ.** β-catenin mediates glandular formation and dysregulation of β-catenin induces hyperplasia formation in the murine uterus. *Oncogene*. 2009;28:31–40.
646. **Mori M, Kitazume M, Ose R, Kurokawa J, Koga K, Osuga Y, Arai S, Miyazaki T.** Death effector domain-containing protein (DEDD) is required for uterine decidualization during early pregnancy in mice. *J Clin Invest*. 2011;121: 318–327.
647. **Tsai CY, Chou CK, Yang CW, Lai YC, Liang CC, Chen CM, Tsai TF.** *Hurp* deficiency in mice leads to female infertility caused by an implantation defect. *J Biol Chem*. 2008;283:26302–26306.
648. **Kim TH, Lee DK, Franco HL, Lydon JP, Jeong JW.** ERBB receptor feedback inhibitor 1 regulation of estrogen receptor activity is critical for uterine implantation in mice. *Biol Reprod*. 2010;82:706–713.
649. **Jeong JW, Kwak I, Lee KY, Kim TH, Large MJ, Stewart CL, Kaestner KH, Lydon JP, DeMayo FJ.** Foxa2 is essential for mouse endometrial gland development and fertility. *Biol Reprod*. 2010;83:396–403.
650. **Li Q, Kannan A, DeMayo FJ, Lydon JP, Cooke PS, Yamagishi H, Srivastava D, Bagchi MK, Bagchi IC.** The antiproliferative action of progesterone in uterine epithelium is mediated by Hand2. *Science*. 2011;331:912–916.
651. **Wang W, Van de Water T, Lufkin T.** Inner ear and maternal reproductive defects in mice lacking the *Hmx3* homeobox gene. *Development*. 1998;125:621–634.
652. **Lee K, Jeong J, Kwak I, Yu CT, Lanske B, Soegiarto DW, Toftgard R, Tsai MJ, Tsai S, Lydon JP, DeMayo FJ.** Indian hedgehog is a major mediator of progesterone signaling in the mouse uterus. *Nat Genet*. 2006;38:1204–1209.
653. **Ernst M, Inglese M, Waring P, Campbell IK, Bao S, Clay FJ, Alexander WS, Wicks IP, Tarlington DM, Novak U, Heath JK, Dunn AR.** Defective gp130-mediated signal transducer and activator of transcription (STAT) signaling results in degenerative joint disease, gastrointestinal ulceration, and failure of uterine implantation. *J Exp Med*. 2001;194:189–203.
654. **Sun X, Zhang L, Xie H, Wan H, Magella B, Whitsett JA, Dey SK.** Krüppel-like factor 5 (KLF5) is critical for conferring uterine receptivity to implantation. *Proc Natl Acad Sci USA*. 2012;109:1145–1150.
655. **Song HS, Lim HJ, Das SK, Paria BC, Dey SK.** Dysregulation of EGF family of growth factors and COX-2 in the uterus during the preattachment and attachment reactions of the blastocyst with the luminal epithelium correlates with implantation failure in LIF-deficient mice. *Mol Endocrinol*. 2000;14:1147–1161.
656. **Daikoku T, Cha J, Sun X, Tranguch S, Xie H, Fujita T, Hirota Y, Lydon J, DeMayo F, Maxson R, Dey SK.** Conditional deletion of Msx homeobox genes in the uterus inhibits blastocyst implantation by altering uterine receptivity. *Dev Cell*. 2011;21:1014–1025.
657. **Nallasamy S, Li Q, Bagchi MK, Bagchi IC.** Msx homeobox genes critically regulate embryo implantation by controlling paracrine signaling between uterine stroma and epithelium. *PLoS Genet*. 2012;8:e1002500.
658. **Mukherjee A, Soyal SM, Fernandez-Valdivia R, Gehin M, Chambon P, Demayo FJ, Lydon JP, O'Malley BW.** Steroid receptor coactivator 2 is critical for progesterone-dependent uterine function and mammary morphogenesis in the mouse. *Mol Cell Biol*. 2006;26:6571–6583.
659. **Mulac-Jericevic B, Mullinax RA, DeMayo FJ, Lydon LP, Conneely OM.** Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform. *Science*. 2000;289:1751–1754.
660. **Lague MN, Detmar J, Paquet M, Boyer A, Richards JS, Adamson SL, Boerboom D.** Decidual PTEN expression is required for trophoblast invasion in the mouse. *Am J Physiol Endocrinol Metab*. 2010;299:E936–946.
661. **Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM, Dey SK.** Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell*. 1997;91: 197–208.
662. **Tranguch S, Chakrabarty A, Guo Y, Wang H, Dey SK.** Maternal pentraxin 3 deficiency compromises implantation in mice. *Biol Reprod*. 2007;77:425–432.
663. **Franco HL, Lee KY, Rubel CA, Creighton CJ, White LD, Broaddus RR, Lewis MT, Lydon JP, Jeong JW, DeMayo FJ.** Constitutive activation of smoothened leads to female infertility and altered uterine differentiation in the mouse. *Biol Reprod*. 2010;82:991–999.
664. **Hirota Y, Daikoku T, Tranguch S, Xie H, Bradshaw HB, Dey SK.** Uterine-specific p53 deficiency confers premature uterine senescence and promotes preterm birth in mice. *J Clin Invest*. 2010;120:803–815.
665. **Dunlap KA, Filant J, Hayashi K, Rucker EB, 3rd, Song G, Deng JM, Behringer RR, DeMayo FJ, Lydon J, Jeong JW, Spencer TE.** Postnatal deletion of Wnt7a inhibits uterine gland morphogenesis and compromises adult fertility in mice. *Biol Reprod*. 2011;85:386–396.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 20 October 2014. at 08:41 For personal use only. No other uses without permission. . All rights reserved.