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# Decidualization and Related Pregnancy Complications

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**Abstract**

Decidualization is the differentiation of endometrial stromal cells into secretory decidual stromal cells. Human decidualization involves some amount of signaling molecules and pathways as well as genetic reprogramming, which is driven by the postovulatory rise in progesterone levels and local cyclic adenosine monophosphate production. Decidualization extends from the primary decidual zone to the secondary decidual zone, and then exits through apoptosis. Evidences support that decidual fibroblasts function as the pool of decidual stromal cells during pregnancy. Decidualization undergoes an acute inflammatory phase, an anti-inflammatory secretory phase to the final recession phase. The decidualization of the inner layer of endometrium, termed decidua, is the most critical determinant of pregnancy success, which can promote placenta formation, modulate immune tolerance, foster resistance to oxidative stress, sense embryo quality, and control labor. Failure to adequate decidualization in terms of hormones, biochemistry, and immunology leads to adverse pregnancy outcomes, including diseases such as preeclampsia, miscarriage, premature labor, repeated implantation failures, and some age-related decline in reproductive capacity. The development of animal models and in vitro culture systems combined with emerging technologies provides a powerful system to explore the mechanism of decidualization. However, decidualization is a dynamic, multi-step process, and translating of current research progress into disease predictions and interventions for pregnancy complications remains to be achieved. The study of periodic regeneration and spontaneous decidualization of the endometrium will be beneficial to the diagnosis and treatment of pregnancy diseases.

**Keywords:** Decidua; Decidualization; Decidual stromal cells; Endometrial stromal cells; Pregnancy complications

## Introduction

Decidualization denotes the transformation of the endometrial stroma into the decidual matrix. The most important feature is the differentiation of endometrial stromal cells (ESCs) into secretory decidual stromal cells (DSCs). The decidualization of the inner layer of endometrium, termed decidua, prepares the uterus for pregnancy by permitting trophoblasts invading into it and remodeling of spiral arteries. Decidua plays important roles in embryonic implantation, placentation, and pregnancy maintenance. Insufficient decidualization leads to abnormal implantation/placentation and ultimately results in adverse pregnancy outcomes. This study is aimed to review recent evidences. We first summarized the remarkable changes during decidualization and the methods of artificial decidualization (AD), then expanded on the genes and energy metabolism involved in decidualization, and finally reviewed the function of decidua and the adverse pregnancy outcomes due to impaired decidualization.

## Remarkable changes during decidualization

Decidualization is the transformation of fibroblast-like ESCs into large, round or polygonal DSCs during the menstrual cycle and pregnancy. In most mammals, decidualization is initiated by embryo implantation. Humans are one of the few mammalian viviparous species that independently initiate decidualization on the implanted embryo in the mid-luteal phase of each cycle.

### *Markers of decidualization*

In human, decidualization is a spontaneous process, which initiates during the secretory phase of the menstrual cycle controlled by ovarian steroid hormones. In this process, ESCs transform into large epithelioid cells and secrete two protein markers: decidual prolactin (PRL) and insulin-like growth factor binding protein 1 (IGFBP1). NODAL-signaling pathway inhibitor left-right determination factor-2 is another factor highly secreted by decidualized cells. Some key transcription factors such as forkhead box protein O1 (FOXO1) and CCAAT/enhancer binding protein-β and secreted products, such as Wnt family

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member 5A (WNT5A), the inhibitor of canonical Wnt signaling, dickkopf-1, and prokineticin-1 are also putative markers of DSCs. Among them, PRL and IGFBP-1 are now widely used markers to assess the differentiation status of human ESCs in culture.[1]

While in mouse, 4 days after fertilization, the attachment of the blastocyst to the uterine epithelium initiates the process of decidualization. PRL family 8 subfamily a member 2 and alkaline phosphatase liver/bone/kidney are marker genes for decidualization.[2] There is also evidence supporting that the process of mesenchymal epithelial transition exists during in vitro decidualization (IVD) of murine or human ESCs, associated with the down-regulation of snail and vimentin expression, and the upregulation of E-cadherin and cytokeratin expression.[3]

## Characteristics of polyploidization

Polyploidization is a hallmark of mature decidual cells, characterized by large mononuclear or binuclear cells with multiple copies of chromosomes, and has been widely recognized in mice. The DNA content of polyploid decidual cells is >4N, while the DNA content of non-polyploid cells is 2N. In mouse models, localized ESC polyploidization occurs at 6–8 days of pregnancy. Specifically, the secondary decidual zone comprises proliferating and differentiated polyploid stromal cells and forms adjacent to the primary decidual zone by day 6. Later, the secondary decidual zone is fully developed, and polyploidy development is gradually spreading by day 7. Then, the cells comprising the primary decidual zone progressively degenerate by apoptosis, and most of them disappear by day 8.[4] However, limited evidence is available regarding the polyploidization in human. A recent article reported that the stromal cells in the secretory phase or decidual cells were mainly mono-nucleated cells. The proportions of multinucleated cells were 1.1% and 3.7%, respectively. Moreover, human stromal cells displayed as mono-nucleated in both of control and decidual groups after IVD for 8 days, and the proportion of tetraploid (4N) in DSCs was similar to that of the control group, revealed by flow cytometry analysis. Thus, the polyploidization of stromal cells is not essential for human uterine decidualization.[5]

Cell cycle molecules, growth-factors, signaling mediators, and homeobox transcription factors have been shown to regulate the formation of polyploidy of decidual cells. Affymetrix gene chip analysis for the whole mouse genome transcripts reveals a total of 1015 up-regulated genes and 1207 down-regulated genes in the polyploid populations, as compared to the non-polyploid group. According to functional enrichment analyses, up-regulated polyploid genes appear to be implicated in cell/nuclear division, adenosine triphosphate (ATP) binding, metabolic processes, and mitochondrial activity, while down-regulated genes are mainly involved in apoptosis and immune processes.[6]

## Decidual fibroblasts: the "pool" of decidual cells

DSCs behave as a proliferative quiescent state, especially for the DSCs in their late stage of decidualization, which is marked by an increase in cellular death through apoptotic pathways.[7] Thus, logically, there should be a supplementary pool for DSCs during the long gestation of pregnancy. Actually, evidences from cultured stromal fibroblasts isolated from human first trimester and term decidua,[8] the dynamic proportion of decidual endothelial cells and the fibroblasts during the peripartum,[9] the process of functional evolution from the pseudotemporal ordering in human first-trimester decidua and peripartum decidua,[9,10] and the decidualization of decidual fibroblasts from human term decidua after in vitro inducing,[11] all support that decidual fibroblasts function as the pool of DSCs during pregnancy.

## Artificial decidualization

Direct probing of decidualization in humans during pregnancy is impracticable due to ethical restrictions and implementation difficulties. Fortunately, even after 75 million years of evolutionary divergence, the uterus of humans and mice still shares a remarkable degree of cellular and molecular conservation.[12] Mouse models of decidualization as well as AD models in human have been thoroughly established and characterized.

## Mouse models of decidualization

Mouse models are powerful tools for improving our understanding of uterine decidualization because mice can be easily manipulated with well-established genetic tools and experimental approaches.[13] There are three well-established mouse models for studying decidualization: natural pregnancy decidualization (NPD), AD, and IVD. Specifically, the NPD model is established by allowing fertile females to mate with fertile males of the CD-1 strain: in the AD model, sesame oil and glass beads are used and uterine scratches stimulate the mouse uterus; the IVD model induces cultured stromal cells by incubation with estrogen plus progesterone. Among them, the NPD model is thought to be the golden standard of mouse decidualization. Through comparative analysis at the molecular level by RNA-seq approach, AD is found a reliable model for mouse decidualization, but IVD model should be optimized to mimic NPD at the transcriptomic level.[14]

## IVD in human

IVD models are practical, and they are useful for studying decidualization relevant mechanisms and avoiding ethical dilemmas in humans. Currently, immortalized human ESCs, primary human ESCs obtained from uterine tissue after surgical resection or curettage, are well established tool cells in research of IVD. In early days, estradiol (E2) combined with progesterone (P4) was often used for inducing decidualization, but it would take 12 to 20 days. Nowadays, the most commonly used induction schemes are E2, combined with P4 and cyclic adenosine monophosphate (cAMP), which is termed the EPC scheme, or without E2, which is termed PC scheme. The effective induction concentration of P4 or medroxyprogesterone acetate ranges from 1 μmol/mL to 1 mmol/mL, and that of E2 is 10 nmol/mL in EPC scheme and of cAMP is 0.5 mmol/mL. With the cAMP-modified induction scheme, the process can be shortened to 3–4 days to observe a good effect.[15]

Moreover, human amnion-derived mesenchymal stem cells and menstrual blood-derived mesenchymal stem cells (MMCs) can be induced to differentiate into endometrial stroma-like cells. After treatment with cAMP, amnion-derived mesenchymal stem cells and MMCs prompt remarkable morphological changes, increase the expression of decidualization markers (PRL and IGFBP1), and attenuate the expression of surface markers unique to MMCs, which provides novel model for decidualization.[16]

During IVD, obvious decidual characteristics can be observed as early as the first day of induction. These morphodynamical changes have been shown to be associated with alterations in cellular behavior and homeostasis.[17] Notably, extensive, dynamic changes in the early (3 days) and late (8 days) decidual cell transcriptomes have been documented in human ESCs in the IVD model.[7] The proteomes of ESC-derived extracellular vesicles following a decidualizing stimulus define the cells' potential for decidualization success.[18] Additionally, there are many differences between human and mouse IVD, which are summarized as follows: (1) only 4 days of IVD time in mice, maybe because the 4-day estrus cycle in mice is much shorter than the 28-day menstrual cycle in humans; (2) primary mouse ESCs can only divide 2–3 times in vitro and a longer time of IVD causes abnormal morphological changes likely due to replicative senescence; and (3) the addition of cAMP analogs (EPC scheme) is no better than E2 combined P4 scheme to induce IVD in mice.[14]

## Genes involved in decidualization

The ability of ESCs to differentiate into DSCs appears to the key element in the decidual transformation. However, DSCs are not simply modified ESCs; they are a distinct cell type resulting from genetic reprogramming of ESCs. Lots of genes are involved in the decidualization process.

### *Signaling molecules and pathways essential for decidualization in mice*

Herein, this part focuses on reviewing the exciting findings about signaling pathways and molecules essential for mouse decidualization, mostly from genetically modified mouse models. Figure 1 displays the major signaling pathways and molecules involved in decidualization.

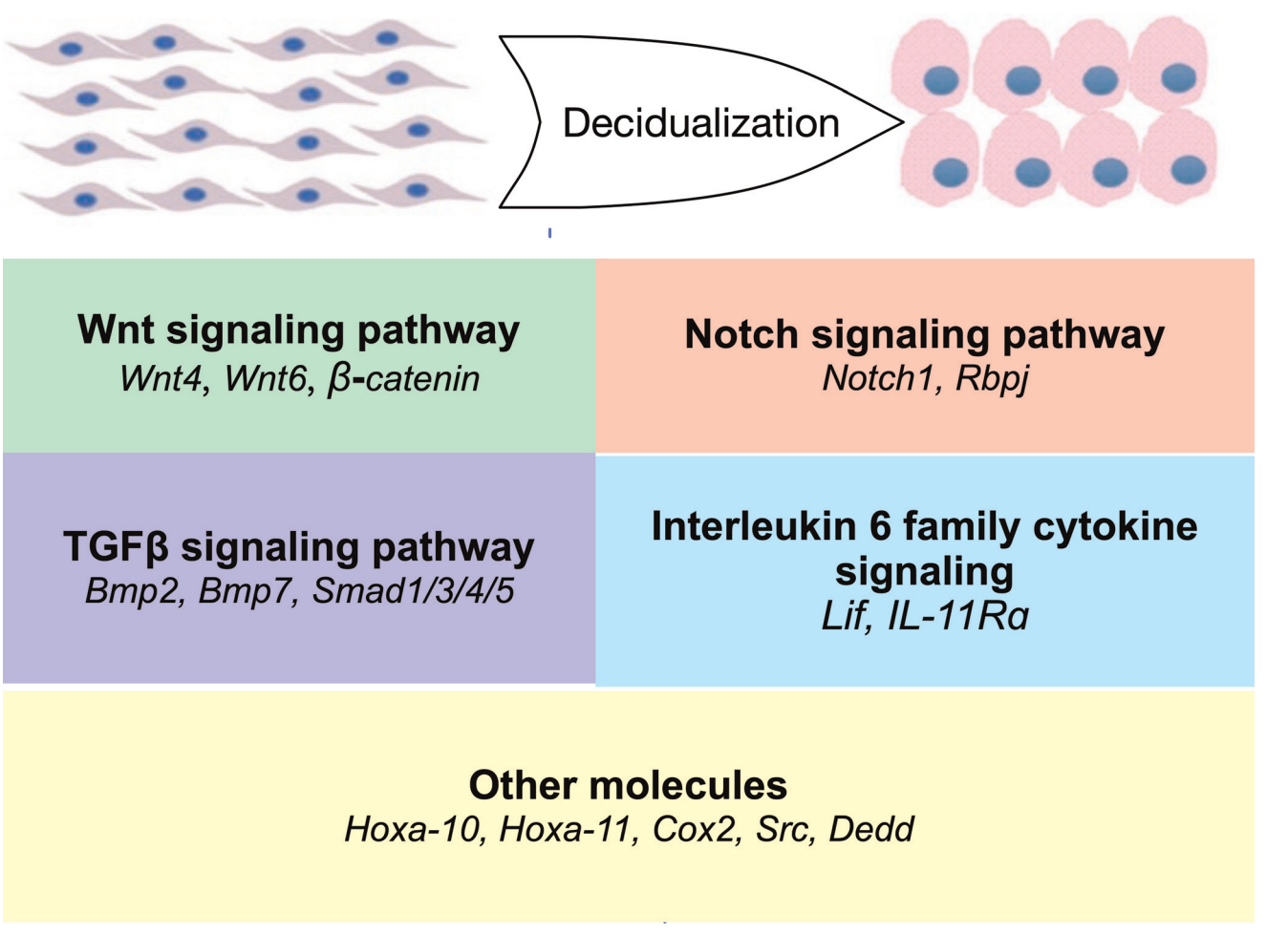


Figure 1. Major signaling molecules and pathways essential for decidualization in mice. Wnt: Wingless-type MMTV integration site family; Wnt4: Wnt family, member 4; Wnt6: Wnt family, member 6; Rbpj: Recombination signal binding protein for immunoglobulin kappa J region; TGFβ: Transforming growth factor-beta; Bmp2: Bone morphogenetic protein 2; Bmp7: Bone morphogenetic protein 7: Smad1/3/4/5: SMAD family member 1/3/4/5; Lif: Leukemia inhibitory factor; IL-11Rα: Interleukin 11 receptor, alpha chain; Hoxa-10: Homeobox A10; Hoxa-11: Homeobox A11; Cox2: Cyclooxyrgenase 2; Src: Rous sarcoma oncogene; Dedd: Death effector domain-containing.

The importance of Wnt signaling pathway in decidualization is underscored by the observations that conditional deletion of *Wnt4*[19] or *Wnt6*[20] in mice results in impaired decidualization. Mice with uterine conditional ablation of *Bmp2*[21] or *Bmp7*[22] mice also exhibit compromised decidualization. Moreover, Smad1/5/4-Amhr2-Cre KO mice also show partially compromised decidualization, which may be caused by the dysregulation of *Bmp2* and *Wnt4*.[23] Additionally, a progesterone receptor (Pgr)-Cre Notch1 bigenic (Notch1d/d) indicated a Notch1-dependent hypomorphic decidual phenotype.[24] Another study confirmed that the deletion of Notch family transcription factor, recombinant signal binding protein-Jκ (RBPJ) in mouse uterus (Pgr cre/+Rbpj flox/flox), leads to subfertility, partly due to decidualization failure.[25] The inhibition of IL-11 signaling attenuates the expression of decidual markers, and the IL-11Rα-deficient mouse model revealed no sustained decidualization after the initial and unimpeded embryo attachment, which results in fetal loss on day 8 of pregnancy.[26] Besides, the injection of a long-acting leukemia inhibitory factor antagonist into the uterus of mice can significantly reduce the extent of decidualization at implantation sites.[27] Other molecules such as homeobox A10,[28] homeobox A11,[29] cyclooxygenase 2 (Cox2),[30] rous sarcoma oncogene,[31] as well as death effector domain-containing protein[32] are also reported to be critical during decidualization process.

### The master regulators and gene expression profiles during human decidualization

Over the past 20 years, the substantial gene expression changes of ESCs during decidualization have been investigated via in vitro models. Human ESCs exhibit complex transcriptional networks through decidualization process. With the development of microarray, RNA sequencing technologies and single-cell sequencing (scRNA-seq), it has become possible to detect molecular changes in tissues and even single cells. During decidualization process, human ESCs spontaneously differentiate into DSCs in response to increased P4 and cAMP.

Human decidualization is precisely regulated by maternal hormones: ovarian estrogen and P4. P4 activates progesterone receptor (PGR) signaling networks and directs the transcription target genes by binding and activating PGR.[33] The cAMP signaling pathway is also activated during P4-dependent decidualization, and then integrates P4 action by sensitizing ESCs to P4 and stimulating the transcriptional activity of PGR.[34]Interleukins such as interleukin-1β (IL-1β),[35] IL-11[36] and leukemia inhibitory factor[27] have been proved to be vital for sustaining decidualization processes. TGFβ superfamily members including TGFβ1,[37] BMP2[38] and left-right determination factor [39] can mediate and maintain decidualization by promoting cAMP and extracellular matrix (ECM) signaling. Additionally, Wnt4 regulates human decidualization via β-catenin signaling pathways.[40] Protein O-fucosyltransferase 1 is found to promote endometrial decidualization by enhancing the O-fucosylation of Notch1.[41]

Non-coding RNAs (ncRNAs) and epigenetic regulation are also involved in human decidualization process. It is reported that 26 microRNAs are upregulated, and 17 microRNAs are downregulated in human DSCs.[42] The competitive endogenous RNA (ceRNA) network has been proved to regulate the decidualization process by our team. It has been suggested long noncoding RNAs (lncRNAs) hexokinase 2 pseudogene 1 (HK2P1), along with its associated gene hexokinase 2 (Hk2) via competing for the shared miR-6887-3p, is necessary for decidualization.[43] LncRNA phosphoglycerate kinase 1, pseudogene 1 (PGK1P1) has also been demonstrated as a ceRNA to regulate phosphoglycerate kinase 1 (PGK1) expression through miR-330-5p and play a vital role in human decidualization.[44]

The mining of gene expression data revealed that human ESCs express 147 genes coding for epigenetic effectors, of which 33 (22%) are significantly regulated upon decidualization. Two-thirds of differentially expressed chromatin-modifying genes are down-regulated during the transition from a proliferative to a differentiated human ESC phenotype.[45] In particular, the knockdown of enhancer of zeste homolog 2 in decidualizing human ESCs resulted in concomitant higher expression of *PRL* and *IGFBP1*, with a reciprocal enhancement of histone acetylation by reducing the level of trimethylated lysine 27 of histone 3.[46]

A scRNA-seq of the human endometrium reveals that the ligand–receptor pairs are upregulated respectively, in ESCs and lymphocytes, including IL15 and interleukin 2 receptor subunit beta, interleukin 2 receptor subunit gamma, major histocompatibility complex class I genes, and NKR. This suggests that decidualization in the natural human menstrual cycle is characterized by direct interplay between lymphocytes and ESCs.[47]

## Energy metabolism during decidualization

Metabolic processes including energy supply and regulatory circuits involved in decidualization process have been intensively studied. This section focuses on reviewing the metabolic pathways, metabolite secretion regulators, and mitochondrial functions associated with decidualization based on existing evidence.

### Metabolic pathways needed during decidualization

Glycolysis pathway is critical for decidualization both in humans and mice. Aerobic glycolysis-related genes and factors including pyruvate kinase M2, glucose transporter 1, Hk2, glucose-6-phosphate dehydrogenase, lactate dehydrogenase A, and pyruvate dehydrogenase kinase, isoenzyme 1 are induced during the IVD of mouse ESCs. Furthermore, the mRNA levels of these genes are significantly induced on day 5 and are all further enhanced substantially on day 8. Similar results have also been found in human ESCs in the IVD models.[43,44] Additionally, lactate shuttle exists between decidual cells and the undifferentiated stromal cells.[48] Silencing the expression of glycolysis-related genes such as pyruvate kinase M2 and Pfkfb3 in mouse ESCs, HK2, and PGK1 in human ESCs, or inhibiting glycolysis in pregnant mice can hinder IVD process or retard deciduoma in mice.[43,44,48,49] G-protein-coupled receptor 120 also promotes and maintains decidualization in human ESCs via regulating glucose metabolisms through improving glucose transporter

1-mediated glucose uptake.[50] The acceleration of the glycolytic flux by steroid receptor coactivator-2 is also essential for endometrial decidualization.[49]

Fatty acid beta-oxidation is also critical for decidualization. It has been reported that reducing the activity of carnitine palmitoyltransferase 1 either by short hairpin RNA-mediated silencing or by treatment with the inhibitor blocks the early decidualization of a human ESC line.[51] Additionally, the knockdown expression of carnitine palmitoyltransferase 1 in human primary ESCs, which is the rate-limiting step of β-oxidation by activating fatty acids, results in a significant diminution of the expression levels of decidual markers after 4 days in decidualization media. There is also in vivo evidence in mice that decidualization requires the activity of the fatty acid β-oxidation pathway.[51]

### *Metabolites regulating decidualization*

A recent study demonstrated that increased level of uric acid in the decidualized endometrium in humans and mice leads to the formation of monosodium urate crystals. Further, monosodium urate can enhance the decidualization response of ESCs by upregulating inflammatory genes such *Ptgs2* and *IL11*.[52] Notably, a latest review described the regulatory roles of lipid metabolites including phospholipid, sphingolipid, and cholesterol metabolites on uterine decidualization through the production of cannabinoids, prostaglandins, lysophosphatidic acid, sphingosine-1 phosphate, and steroid hormones.[53] Other metabolic factors such as vitamin D deficiency and endoplasmic reticulum stress can also influence decidualization. In addition, the creatine kinase circuit serves as an immediate temporal energy buffer and maintains ATP turnover, which is particularly important for cells with high or fluctuating energy demands. The creatine metabolism is up-regulated in human endometrial tissue during the secretory phase of the menstrual cycle. Therefore, the activity of cytosolic creatine kinase may be necessary for regulating energy homeostasis during decidualization by producing ATP from phosphocreatine stores.[54]

Another study analyzed time-resolved changes in metabolite secretions (exometabolomic) of decidualized human primary ESCs over eight days and found that 79 annotated metabolites differentially secreted upon decidualization, including prostaglandin, sphingolipid, and hyaluronic acid metabolites. These secreted metabolites encompassed 21 metabolic pathways: the most prominent ones are glycerolipid and pyrimidine metabolites.[55] However, this is just the first step to highlight the potential of secreted metabolites acting as autocrine or paracrine signaling molecules as the decidualization process unfolds.

### *Mitochondrial activity is vital for decidualization*

Many mitochondrial genes including *Me1*, *Eln*, *Ak1*, *Tmtc1*, *Acss3*, *Tfrc*, *Abat*, and *Limk2* exhibit upregulation patterns in mouse polyploidy decidual cells. Mitochondrial perturbation causes the attenuation of decidual cell polyploidy, which is crucial for appropriate control of uterine decidualization.[6] It has been reported that other molecules influencing mitochondrial function or participating in energy metabolism can also regulate decidualization process. Folate deficiency can inhibit the apoptosis of decidual cells via the mitochondrial apoptosis pathway, thereby restraining decidualization of the endometrium.[56] There is new evidence that ATP signaling cascades are involved in decidualization. Extracellular ATP can directly promote the decidualization of human ESCs *via* P2Y-purinoceptors.[57]

## Functions of DSCs and decidua

Decidualization confers unique characteristics of the endometrium that are crucial for the formation of the placenta, including the ability to regulate the invasion of trophoblasts, to modulate local vascular and immune responses, and to resist environmental and oxidative stress. In addition, decidua is crucial for detecting embryo quality and responding accordingly and regulating labor initiation. This section briefly reviews these contents.

### *Placenta formation*

Decidua is a specialized tissue composed of DSCs, glands, immune cells, blood, and lymph vessels. In the process of decidualization, the DSCs controlled by hormones (mainly $E_2$ and P4) acquire specific functions related to the recognition, selection, and acceptance of the embryo as well as the maternal immune tolerance. $E_2$ and P4 modulate the development and function of uterine vascular system, resulting in drastic changes in the volume, elasticity, and nutrient transportation of the entire uterus. Specifically, $E_2$ has greater impact on vascular permeability via suppressing adhesion molecules such as E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1. P4 has more angiogenic effects via inducing proliferative factors from endometrial cells, such as vascular endothelial growth factor (VEGF), angiopoietin-2, and fibroblast growth factor 2.[58]

The important feature of decidua is its control of trophoblast invasion to access the maternal blood supply, but not to the extent of endangering the mother. DSCs express matrix metalloproteinases (MMPs) to assist in ECM degradation and further enhance trophoblast invasion. On the other hand, DSCs also antagonize MMP activity by producing tissue inhibitors of metalloproteinases, and consequently blocking trophoblast invasion.[59] Overall, decidual cells balance the secretion of MMPs and tissue inhibitors of metalloproteinase, control trophoblast cell migration, and prevent an aggravated invasion under tight regulation following a strict balance. Thus, decidualization prepares the uterus for embryo implantation by inducing gradual and profound alterations in gene expression, cellular functions, and tissue remodeling until the placenta is fully formed.[60]

### *Immune modulator*

Maintaining immune tolerance is another function of maternal decidua. Pregnancy hormones, the decidualization process, and the interactions of DSCs and trophoblasts with immune cells contribute to the establishment of pregnancy-related immune homeostasis. However, DSCs might be the main operational immune modulators that are able to alter lymphocyte function and suppress immune

responses. Decidual NK (dNK) cells are the predominant immune population and accounting for approximately 70% of all leukocytes. During the first trimester of pregnancy, dNK cells interact with the human leukocyte antigen ligand expressed on extravillous trophoblasts and mediate the immune tolerance at the maternal-fetal interface, resulting in noncytotoxic response against the semi-allogeneic embryos. In addition, they are key regulators in early pregnancy because they play a fundamental role in vascular remodeling, trophoblast invasion, and embryonic development by secreting several cytokines.[61] However, DSCs can secrete IL-8, which drives $CD34^+$ precursor cells differentiating into mature NK cells.[62] DSCs can also suppress the proliferation of IL-15 activated NK cells and inhibit their granzyme A and perforin synthesis. Moreover, DSCs can weaken the NK cell cytotoxicity and decrease the lysis of erythroleukemia and melanoma target cells by secreting the immune-suppressive factors PGE2 and indoleamine 2,3-dioxygenase.[63]

Various antigen-presenting cell (APC) populations are observed in human decidua during pregnancy, the majority of which are macrophages, while the much smaller part is mature dendritic cells (DCs). Although decidual APCs contact with different immune cells, their function is unclear. Notably, DSCs support decidua-specific microenvironment and modify the functions of infiltrating APCs. DSCs can modulate the DC maturation process; specifically, they inhibit the differentiation of blood monocytes into immature DCs ($CD14^+CD1a^+$) by 50%–96% via secreting IL-14 and granulocyte macrophage colony-stimulating factor cytokines. DSCs can also inhibit the differentiation of immature DCs into mature DCs via secreting macrophage inhibitory cytokine-1.[64,65]

### Resistance from oxidative stress

The fetal-maternal interface, defined as the interaction between the decidualized endometrium and invasive extravillous trophoblast cells, induces large amounts of intracellular reactive oxygen species and oxidative stress by exposing to extensive changes in oxygen tension during pregnancy. However, DSCs have the ability to encapsulate and enclose the embryo, and their noteworthy resistance to oxidative cell damage assures that the pregnancy is protected against these environmental stressors.[66]

First, decidualization is associated with the induction of antioxidant enzymes, including copper-zinc superoxide dismutase located in the cytosol and manganese superoxide dismutase located in the mitochondria.[67] Second, the expression of serum- and glucocorticoid-inducible kinase-1 is increased in DSCs, which can safeguard the decidual-placental interface against oxidative stress signals.[68] Third, the differential expression of forkhead transcription factor FOXO1 and FOXO3a confers resistance to oxidative cell death upon decidualization.[69] These findings suggest that endometrial decidualization regulates embryo invasion and tissue homeostasis and presents resistance to oxidative stress.

### Sensor for embryo quality

In contrast to most species, the human endometrium decidualizes in response to endocrine rather than embryonic cues. The purpose of the emergence of cyclic decidualization in the absence of embryos is to prevent inappropriate investment in invasive but poorly viable embryos. This emerging concept of decidua as a biosensor for embryo quality is first observed in a human in vitro embryo/DSC coculture system in 2010. Surprisingly, the presence of a developing embryo showed no significant effect on decidual secretions, apart from a modest reduction in IL-5 levels. In contrast, arresting embryos triggered a strong response characterized by selective inhibition of key implantation mediators and immunomodulators including IL-1β, -6, -10, -17, and -18, eotaxin, and heparin-binding EGF like growth factor. Further, repeated co-cultures with undifferentiated ESCs, but none of the secreted cytokines were affected by the presence of a developing or arresting embryo.[70] Flushing of the mouse uterus with culture medium from low-quality human embryos triggers a stress response akin to that observed in primary human DSCs. Moreover, this research demonstrates that developmentally impaired human embryos elicit an endoplasmic stress response in human decidual cells. Moreover, a pivotal role for dNK cells in embryo biosensing and endometrial fate determination at implantation has been proved. Exposure to medium conditioned by successfully implanted embryos does not inhibit the ability of dNK cells to kill senescent decidual cells, while those from embryos that failed to implant do.[71]

### Labor initiation

Decidua is essential not only in the establishing and maintaining of pregnancy, but also controls labor initiation. Prostaglandins (PGs) are well-known factors involved in the onset of labor, both at term and preterm. It is found that the endogenous levels of PGs in the decidua are 200-fold lower in pregnancy than those in the endometrium at any stage of the menstrual cycle, which is caused by a decrease in PG synthesis. The administration of exogenous PGs at any stage of gestation can induce abortion.[72] Recent evidence supports that the decidua is the primary organ tissue that determines labor initiation, as the "decidual clock" rather than the "placental clock" controls the timing of labor initiation. Norwitz *et al.*[73] reviewed the molecular regulation of parturition in 2015 and outlined a hypothesis, which refers to the inhibited inflammation (primarily through PGs) in the decidua is critical for pregnancy maintaining, and advancing gestational age is associated with a withdrawal of active suppression of the decidua to signals capable of inducing inflammation. Further, a recent review states that inflammatory amplification is the central tenet of uterine transition for labor. The amplification events occur because of uterine activation and functional progesterone withdrawal. Before labor approaches, the decidua upregulates uterine activation proteins and undergoes activation as shown below: (1) COX2, which is a major producer of PGs, is considerably elevated in the decidua in late gestation; (2) The expression levels of uterotonic oxytocin receptor and decidual PGF-2a receptor increase in decidua; (3) The expressions of all PGR isoforms diminish; (4) Pro-inflammatory mediators such as IL-6, CCL2, CCL4, CCL5, CXCL8, and CXCL10 are upregulated in the decidua at term and correlate with decidual leukocyte abundance.[74] Notably, the senescence of the decidual

tissue turned out to be essential for the initiation of labor.[75] Additionally, DSCs are heterogeneous at the transcriptomic level at different stages of pregnancy.[10,76,77] DSCs may maintain their heterogeneity during the perinatal period until delivery, which may have important implications for labor. New evidence from scRNA-seq has also highlighted the heterogeneity of peripartum DSCs, which play multiple roles in labor.[78]

## Impaired decidualization and adverse pregnancy outcomes

Adverse pregnancy outcomes refer to the disorders that seriously affect the health of pregnant women and the fetuses. Their etiology is complex, and many of them are associated with deficient decidualization. The key genes involved in decidualization of several pregnancy complications are listed in Table 1.

### *Preeclampsia (PE)*

PE is a pregnancy-specific complication characterized by new-onset hypertension, which occurs after 20 weeks of gestation.[79] It is a major cause of maternal and perinatal mortality and morbidity, and complicates 5%–8% of all pregnancies worldwide.[80] Research on isolated human ESCs from nonpregnant participants with a previous severe PE history finds that cultured cells fail to be decidualized by analyzing PRL and IGFBP1 compared with the control group.[81] The deficient decidualization of PE exist a long time, and the impairment of decidualization might begin before pregnancy.[82] In this regard, decidualization defects caused by the dysfunctions of decidual cells seriously affect the normal biological processes of embryo implantation and pregnancy maintenance.

The transcriptome changes a lot in the decidua of PE, which is revealed in a study by using RNA-seq. A total of 1652 lncRNAs are found differentially expressed in the decidua of PE, among which aerobic glycolysis pathway has attracted attention.[83] The two glycolysis enzymes (HK2, PGK1) and their LncRNA (HK2P1, PGK1P1, PGK1P2), as a pair of ceRNAs, have been proved to play a crucial role in human decidualization by regulating the glycolysis pathway and angiogenesis.[43,44] In addition, abnormal proliferation and differentiation affect the normal growth and development of decidual cells, thus affecting the invasion of trophoblast cells into decidual cells, which in turn contribute to the occurrence of PE. Studies have revealed that *WNT5A* and *WNT4* are downregulated in the decidual tissues of PE. The downregulation of *WNT4* and *WNT5A* can inhibit the differentiation of ESCs, participating in the occurrence and development of PE.[84] Moreover, reduced decidual cell apoptosis by inhibiting the mechanistic target of rapamycin kinase (mTOR) signaling pathway will affect the invasion of PE trophoblasts. The decreased expression of DNA damage induced transcript 4 in decidual tissues is likely associated with PE and impaired decidualization. In addition, DNA damage induced transcript 4 can mediate apoptosis through mTOR during decidualization to regulate the invasion of trophoblastic cells.[85] In another study, BCL2 interacting protein 3, which is considered as a regulator of apoptosis in many tumors, is also found to be downregulated in the decidua of PE. BCL2 interacting protein 3 regulates the apoptosis of decidual cells through the mTOR/P70S6K/Bcl-2 signaling pathway, thereby participating in the pathogenesis of PE.[86] Furthermore, the abnormal gene expression profiles of decidual macrophages may cause abnormal export (antigen-presenting) function, which affects the interaction between decidual macrophages and other immune cells, thereby hindering the original immune regulation, and ultimately taking part in the occurrence of PE.[87] In addition, the lower expression of estrogen receptor α, hepatocyte growth factor, and CD206 has also been proved to lead to the imbalance of different inflammatory macrophages, thereby contributing to the pathogenesis of PE.[88]

Finally, angiogenesis plays an important role not only in embryo implantation, but also in decidual angiogenesis in the early post-implantation period. The expression of COX2 is significantly decreased in the decidua of PE. COX2 in the decidua may affect the occurrence of PE through VEGF.[89] Zhang *et al.* showed that the transcription factors specificity protein 1 (SP1) and E1A binding protein p300 (P300) are downregulated in severe PE decidua, and the silence of SP1 and P300 in human ESCs can cause the downregulated expression of VEGF, which may ultimately contribute to the development of PE.[90]

**Table 1**

**Expression of key genes in impaired decidualization of pregnancy complications.**

| Pregnancy complications | Key genes | Expression pattern | References |
|---|---|---|---|
| PE | *BNIP3, BNIP3P1* | Down-regulated | 86 |
| | *COX2* | Down-regulated | 89 |
| | *DDIT4* | Down-regulated | 85 |
| | *ERα, HGF, CD206* | Down-regulated | 88 |
| | *HK2, HK2P1* | Down-regulated | 43 |
| | *PGK1, PGK1P1* | Down-regulated | 44 |
| | *SP1, P300* | Down-regulated | 90 |
| | *WNT4, WNT5A* | Down-regulated | 84 |
| RPL | *IGF1* | Down-regulated | 97 |
| | *RANKL* | Down-regulated | 98 |
| PTB | *p53* | Down-regulated | 102,103 |
| | *TLR4* | Down-regulated | 104 |
| | *GATA2, HAND2* | Down-regulated | 105 |
| RIF | *COX2* | Down-regulated | 110 |
| | *HOXA10* | Down-regulated | 111,112 |

BNIP3: B-cell lymphoma 2/adenovirus E1B 19KD interacting protein 3; COX2: Cyclooxygenase 2; DDIT4: DNA damage inducible transcript 4; ERα: Estrogen receptor α; GATA2: GATA binding protein 2; HAND2: heart and neural crest derivatives expressed 2; HGF: hepatocyte growth factor; Hk2: hexokinase 2; HK2P1: Hexokinase 2 pseudogene 1; HOXA10: Homeobox A10; IGF1: Insulin-like growth factor 1; P300: E1A binding protein p300; PE: Preeclampsia; PGK1: Phosphoglycerate kinase 1; PGK1P1: Phosphoglycerate kinase 1, pseudogene 1; PTB: Preterm birth; RANKL: Receptor activator for nuclear factor-κ B ligand; RIF: Repeated implantation failure; RPL: Recurrent pregnancy loss; SP1: Specificity protein 1; WNT5A: Wnt family member 5A.

### *Miscarriage*

Miscarriage refers to the natural death of an embryo or a fetus before it can survive independently. Recurrent pregnancy loss also termed as recurrent spontaneous abortion or recurrent miscarriage (RM), is defined as more than two spontaneous abortions of the same couple.[91]

Decidualization deficiency resulting in the lack of intimal secretion and the change of local immune environment has always been considered to be an important cause of spontaneous abortion.[92] The ESCs of RM patients show an aberrant decidualization.[93] In addition, a series of genes related to decidualization are found abnormally expressed in the decidua between patients with spontaneous abortion or recurrent abortion.[94]

Further, the endometrium of patients with recurrent abortion has defects in the recognition of embryo quality, permitting the implantation of poor-quality embryos; when the subsequent embryonic development broke down, miscarriage occurs. According to a scratch experiment, the migration of ESCs in normal pregnant women is inhibited in the presence of low-quality embryos compared with high-quality embryos, while there is no difference in the migration of ESCs in RM patients. For example, in the presence of AC-1 M88 trophoblast cell line derived spheroids, the migration of ESCs from women with RM is enhanced, indicating that the ESCs of RM women cannot discriminate the differences between the embryos and trophoblast spheroids.[95] Apart from function as a sensor of embryo selection, the local regulatory effect of decidua on the immune environment is also found to be related to miscarriage. The autophagy of ESCs and retention of dNK cells are insufficient in patients with unexplained spontaneous abortion.[96] The decidualization of ESCs also reduces the cytotoxicity of dNK through wisp2/IGF1 signaling pathways. In RM patients, IGF-1 expression is decreased, making the cytotoxicity of dNK cells out of regulation and results in an increased secretory level of proinflammatory cytokines.[97] Further, the expression of receptor activator for nuclear factor-κb ligand (RANKL) in ESCs as well as of the RANKL receptor in decidual dγδT cells of RM patients is lower. The abnormal low level of RANKL/RANK limits the communication between ESCs and dγδ T cells and suppresses the polarization of dγδ T cells which leads to pregnancy loss.[98] Additionally, it reveals a conspicuous link between the pro-senescent decidual response in peri-implantation endometrium and recurrent pregnancy loss by reconstructing the decidual pathway at a single-cell level in vitro. In the absence of immune cell-mediated clearance of senescent decidual cells, secondary senescence transforms decidual cells into progesterone-resistant cells that abundantly express ECM remodeling factors.[99]

## Preterm labor

Preterm birth (PTB) is one of the most common causes of perinatal mortality. It refers to delivery before 37 completed weeks of gestation.[100] PTB is the prime cause of death for children aged younger than 5 years of age and imposes a serious burden on society.[101] Since the decidua functions as labor initiation, the dysfunction of decidualization may cause the occurrence of PTB.[73] In mice, the uterine specific loss of p53 leads to PTB because of premature uterine senescence through a COX2/PGF synthase/PGF-2a pathway.[102] Another study also demonstrates that decidual senescence is associated with PTB through the p53-mTOR-p21-COX2 axis.[103] Deng *et al.* found that mice with endothelial Tlr4 deletion are resistant to lipopolysaccharide-induced PTB, while TLR4 in decidua is vital for perception inflammation during pregnancy.[104] The expression of two transcription factors, GATA binding protein 2 and heart and neural crest derivatives expressed 2, in ESCs may impair the transcriptional programs that regulate the timing of delivery, which may lead to PTB.[105] These results imply that decidual senescence is related to PTB. Besides, one study reported that 39% of early (20–28 weeks) PTB patients and 28% of PTB patients at 28–34 weeks have decidual bleeding.[106] Decidual hemorrhage and inflammation are major causes of PTB. The perivascular decidualized stromal cells promote endometrial hemostasis during placentation. Abnormal endometrial bleeding causes excessive local thrombin production, promotes the infiltration of MMP and neutrophils in decidual cells, and inhibits PR expression, which contributes to adverse pregnancy outcomes such as PTB.[107]

## Repeated implantation failure

Repeated implantation failure (RIF) refers to the cases that young women under the age of 40 fail to obtain a clinical pregnancy after the transferring of high-quality embryos for more than three times.[108] The decidualization in RIF patients is poor, manifested mainly in the reduced decidual secretion, impaired transformation, and limited trophoblast expansion.[109] About 85% of RIF patients have a down-regulated COX2 expression in endometrium.[110] Besides, the downregulation of HOXA10 accompanied with an enhanced HOXA10 SUMOylation is found in the endometrium of RIF.[111,112] Moreover, some genes influencing decidualization and embryo implantation by regulating transcription factor, oxidative stress, and cell proliferation cycle have been found to be differentially expressed between RIF patients and normal women in the endometrium.

## Age-related reproductive decline

The negative impact of maternal age on reproductive outcomes is most commonly associated with the exponential increase in meiotic defects in the oocyte. However, in the absence of karyotype abnormalities, in both humans and mice, decreased reproductive capacity and many pregnancy complications as well as birth defects often occur in elderly mothers. For most of the last century, the endometrium was viewed as the sole effector organ completely controlled by the hypothalamic–pituitary–gonadal axis. A study on mouse model aiming to distinguish fetal from maternal effects on embryo development shows that pregnancy in old female mice is associated with a dramatic increase in developmental variability and severely mis-developed placentas. However, when embryos from old mothers are transferred to young recipients, the development of both embryo and placenta largely returns to normal. This study highlights the importance of the aging uterine environment as a prevalent cause of reproductive decline in older females, but the underlying molecular changes remain to be established.[113]

## Conclusions and perspective

With the rapid increasing of our understanding of decidualization, its importance and its relationship with

pregnancy complications have attracted more and more attention. Therefore, this review gives an integrated summary and discussion and puts forward that decidual fibroblasts function as the pool of DSCs during pregnancy. Animal models, cell lines, and primary ESC and DSC culture systems, combined with emerging technologies, such as scRNA-seq, provide a powerful system to explore the mechanism of decidualization. However, translating all this information into disease predictions and interventions to prevent pregnancy complications remains to be achieved. Decidualization is a dynamic, multi-step process involving the transition from the initial stage of acute inflammation to the anti-inflammatory secretion stage, and finally the recession stage triggered by embryo-induced cellular stress, a decrease in progesterone levels, senescence, or a combination of these processes. Therefore, pre-implantation events or interventions will have a profound impact on the subsequent pregnancy trajectory and outcome.[1] Due to the existence of menstrual cycle in humans, the endometrium has a cyclic disposal and renewal of its superficial layer, which leads to physiological tissue damage. This plasticity of the uterus makes it inherently difficult to predict the likelihood of a successful pregnancy. However, markers of uterine plasticity may help stratify high-risk patients to increase the likelihood of a successful pregnancy. Research on periodic endometrial regeneration and spontaneous decidualization will be beneficial to the diagnosis and treatment of pregnancy diseases.

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## Conflicts of Interest

None.

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