Published in final edited form as:

Placenta. 2017 December ; 60(Suppl 1): S20–S26. doi:10.1016/j.placenta.2017.07.003.

Hormone Dependent Uterine Epithelial-Stromal Communication for Pregnancy Support

Xiaoqiu Wang, San-Pin Wu, and Francesco J. DeMayo*

Reproductive and Developmental Biology Laboratory, National Institute of Environmental Health Sciences, National Institute of Health, Research Triangle Park, NC 27709

Abstract

Human fertility is a relatively inefficient process. Despite the presence of visibly healthy embryos, 30% of pregnancies generated by assisted reproductive technology (ART) fail before the second trimester. The uterine microenvironment plays a critical role in establishing and maintaining a successful pregnancy that requires coordinated communication between the epithelial and stromal cells of the endometrium. The epithelial cells must cease proliferation and become permissive for the conceptus (embryo and associated extraembryonic membranes), while the stromal cells undergoes mesenchymal-to-epithelioid transformation to form the decidua in support of subsequent embryo development. The ovarian steroids Estrogen (E2) and Progesterone (P4) are the major hormones governing these processes. These hormones act via their nuclear receptors, the estrogen receptor, ESR1, and progesterone receptor, PGR, to direct the transcription of genes that orchestrate epithelial and stromal cell communication. This review will discuss the molecular mechanisms utilized by steroid hormones that regulate uterine receptivity, as well, establish and maintain pregnancy.

Keywords

progesterone; pregnancy; uterine receptivity

Introduction

Infertility affects 6.7 million women in the U.S. and 48 million women worldwide [1]. Although assisted reproductive technologies (ART), such as *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) can supply embryos, clinical evidence has shown that at least 30% of pregnancies still cease before the second trimester [2]. This failure is because a successful establishment and maintenance of pregnancy is not only dependent upon the appropriate development of a healthy conceptus, but also dependent upon the ability of the uterus to support the implanting conceptus [3–5]. The ability of the uterus to

*Correspondence: Francesco J. DeMayo, Ph.D., Reproductive and Developmental Biology Laboratory, National Institute of Environmental Health Sciences, 111 TW Alexander Drive, Mail Drop: C1-10, Research Triangle Park, NC 27709, Phone: +1-919-541-0280, francesco.demayo@nih.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

support pregnancy requires the uterus to be receptive for conceptus attachment and invasion and be able to support and protect the developing blastocyst during pregnancy [6, 7]. The ovarian hormones estrogen (E2) and progesterone (P4), acting through their cognate nuclear receptors ESR1 and PGR are critical for this process [3, 4, 8–10]. The mechanisms by which ESR1 and PGR regulate the ability of the uterus to support pregnancy has been investigated *in vivo* by utilizing genetically engineered mouse models [11–14]. The genes associated with uterine function to establish pregnancy have been extensively reviewed [7, 15–20]. Here, we will discuss the process of uterine receptivity and how genetically engineered mouse models have helped to identify the molecular mechanisms governing the ability of the uterus to support pregnancy, mainly based on our previous findings.

1. The window of receptivity

The uterus will only allow a blastocyst to attach and establish pregnancy during a finite period of time termed the window of receptivity [9]. In humans, this period lasts longer and occurs 7–10 days after ovulation during the mid-secretory phase of the menstrual cycle [7]. In domestic animals, the uterus becomes receptive on day 13 in pigs, day 16 in sheep and day 20 in cows [21, 22]. In rodents, the window of receptivity is a short period on the fourth day of pregnancy or pseudopregnancy [20]. Prior to the window of receptivity, conceptuses cannot attach to the uterine epithelial cells, while the uterus is refractory to conceptus attachment after this period. The coordination of the timing of embryo development and uterine receptivity ensures that only healthy conceptuses can initiate pregnancy in a uterine environment that will support the developing fetus. As shown in Figure 1, this process or uterine receptivity requires: 1) uterine epithelial cell proliferation to cease; 2) uterine epithelia cells to become permissive for conceptus attachment (domestic animals) and/or invasion (rodents and humans); and 3) stromal cells to undergo a differentiation termed decidualization which protects and supports the invading conceptus (rodents and humans) [9, 10, 12]. In humans, stromal decidualization occurs recurrently as part of the menstrual cycle (28–30 days) and is a blastocyst-independent process, whereby decidual reaction initiates near the spiral arteries and spreads throughout the endometrium [23]. In mice, decidual reaction is dependent upon mechanical stimulation, whereby the decidualization initiates at the site of blastocyst attachment and then spreads throughout the implantation chamber [24]. The coordination of conceptus development and the uterine window of receptivity is controlled by the ovarian steroids.

2. Ovarian steroid hormones

The timing of the window of receptivity is achieved by the ovarian hormones, E2 and P4 regulating both gene expression in the epithelial and stromal cells of the uterus, as well as the paracrine cross between these compartments [9]. The ovarian secretion of these hormones is coordinated with menstrual cycle (humans and non-human primates) or the estrus (rodents, sheep, cow and pigs). As the counterpart of menstrual cycle, the estrous cycle, particularly in mice last 4–5 days and can be divided into 4 phases: proestrus, estrus, metestrus and diestrus. This cycle lacks a prolonged luteal phase unless there is copulation with a male. In proestrus, prior to ovulation, the ovary accumulates a mature pool of follicles ready for ovulation that occurs during the subsequent estrus phase. Female mice are most

receptive to mating during the estrus phase. A preovulatory surge of E2 acts upon the uterine epithelial cells to stimulate proliferation after mating, termed gestational day (GD) 0.5; E2 decreases by GD1.5. At GD2.5, the formation of a corpus luteum CL results in the production of P4 and maximum expression of progesterone receptor (NR3C3 or PGR) in the uterine glandular (GE) and luminal (LE) epithelia [9]. A nidatory E2 spike at GD3.5 is vital for inducing uterine receptivity [25, 26], due to coordination of stromal cells to undergo decidualization. Similarly, the window of receptivity in human opens on day 19 or 20 of the menstrual cycle after a nidatory E2 spike and lasts for 4–5 days [27]. In rodents, domestic animals and humans, sustained progesterone signaling is critical for uterine receptivity and the maintenance of pregnancy. Ovarian steroid hormones govern uterine receptivity via their associated receptors, i.e. the estrogen receptors (*Esr1* and *Esr2*, transcribed from two genes) and the progesterone receptor (*Pgr* with two isoforms, *PgrA* and *PgrB*, transcribed from the same gene), that elicit transcription activation of downstream target genes that are necessary for a healthy pregnancy or normal menstrual cycle [7, 28].

2.1. Estrogen receptor

Although there are two estrogen receptor genes *Esr1* and *Esr2*, normal uterine physiology is governed by ESR1. The *Esr1*-null female mouse exhibits 1) mating behavior disorder; 2) hypoplastic uteri that are unresponsive to estrogen stimulation; and 3) abnormal ovaries that display cystic and hemorrhagic follicles and the absence of a corpora luteum. On the other hand, ESR2 is only robustly expressed in the ovary [29], oviduct or mammary gland under normal conditions. Functionally, the *Esr2*-null female mouse displays compromised ovulation, and therefore, subfertility. However, no uterine phenotype is observed. Taken together, these findings indicate that ESR1 is critical for the promotion of epithelial proliferation and conceptus attachment [30].

The mechanism by which ESR1 regulates conceptus attachment is through the regulation of the cytokine leukemia inhibitory factor (LIF, encoded by *Lif*). LIF expression is regulated by E2 through the action of ESR1 [31]. LIF expression is induced in the uterine glands during the second nidatory peak of E2 [32]. LIF binds to its receptor, LIFR, in the uterine epithelial cells, which then interacts with its co-receptor, gp130, to phosphorylate STAT3 and initiate translocation to the nucleus to regulate target genes directly responsible for controlling the attachment of the conceptus [32]. Ablation of *Lif* results in infertility due to failure of conceptus attachment and a reduced decidual response. It was demonstrated that a single LIF injection was sufficient to replace the nidatory E2 surge at the time of implantation [31]. This confirms that the function of E2 on the day of implantation is to initiate the induction of LIF and the cascade of events ultimately results in the implantation of the conceptus. This important cytokine was quickly identified as functionally conserved in humans [33]. Epithelial specific ablation of *Esr1* demonstrates that E2 regulation of LIF is due to ESR1 expression in the uterine epithelial cells [30]. On the other hand, unlike the direct regulation of *Lif* by ESR1 in the uterine epithelial cells, the ability of ESR1 to regulate uterine epithelial proliferation is not due to E2 acting directly through epithelial ESR1 but through E2 acting through its receptor in the endometrial stromal cells. This work was first demonstrated by reconstitution of combinations of *Esr1* knockout and wild type epithelial and stromal cells in xenograph transplants to the kidney capsule of immunocompromised

mice [34]. This was later confirmed by epithelial specific ablation of *Esr1* in which the uterine epithelial cells showed E2 induction of proliferation in the absence of ESR1 in the epithelial cells [13]. In addition to ESR1, PGR is critical for the regulation of the window of receptivity and maintenance of pregnancy.

2.2. Progesterone Receptor

The steroid hormone P4 acts via PGR to elicit transcription activation of downstream target genes that are necessary for a healthy pregnancy or normal menstrual/estrous cycle [7, 28]. In humans, domestic animals and rodents, PGR consists of two isoforms, PGR-A and PGR-B, arising from alternative promoter usage in the same gene [35]. Since both PGR isoforms exist in the murine uterus, the total *Pgr* knockout (PRKO) resulted in the disruption of both *PgrA* and *PgrB* transcription [36]. Both female and male mice exhibit normal viability, yet the female PRKO mice are sterile with multiple defects, including 1) aberrant lordosis; 2) uterine failure to undergo decidualization; 3) compromised ability to inhibit E2 signaling; 4) inability to ovulate; and 5) deficiencies in ductal branching during mammary gland development [36]. To further dissect the function of the individual isoform of PGR, site directed mutagenesis using homologous recombination in ES cells to disrupt the translational start sites of *PgrA* and *PgrB* was utilized. This generated mice with ablation of *PgrA* (PRAKO) [37] and *PgrB* (PRBKO) [37]. The PRAKO mice exhibit infertility with similar uterine defects to PRKO mice [37]. Interestingly, the PRAKO mouse uterine epithelial cells are induced to proliferate in the presence of P4, highlighting a unique role for *PgrB* in the regulation of the mouse uterine epithelial cell cycle [37]. The PRBKO mice are fertile with no reported uterine defects; however, ductal failure to undergo proper branching during mammary gland development is present [38]. These results identify functional differences between these two isoforms in response to P4 in the regulation of uterine epithelial proliferation during early pregnancy, and indicates that the PGR-A isoform is the predominant functional isoform in mouse uterus. Not only are the isoforms and levels of PGR critical for uterine function, but also the timing of PGR expression in the various compartments is important for uterine receptivity.

2.2.1. Timing of PGR expression—During pregnancy, PGR is first expressed in the uterine epithelial cells. As pregnancy proceeds, PGR expression is downregulated in the uterine epithelial compartment prior to conceptus implantation while being expressed in the uterine stromal cells [28]. The loss of epithelial expression of PGR during the window of receptivity is most likely due to the downregulation of PGR by its ligand, P4. The importance of this downregulation of PGR prior to conceptus implantation has been investigated by the generation of a mouse model which constitutively expresses PGR-A (*mPgrA^{LsL/+}*) in the uterine epithelial cells (*Wnt7a^{Cre/+}*) [14]. This mouse is infertile with defects in conceptus attachment and stromal decidualization. These mice show a maintained expression of the morphogen, Indian hedgehog, *Ihh*, as well as, reduction in the cytokine, leukemia inhibitory factor (LIF), a key regulator of conceptus receptivity, prior to the receptive period. Further transcriptomic and cistromic analyses of the mouse uterus at GD4.5 identified the PGR-binding events in regions upstream of the *Lif* gene, indicating PGR-A may inhibit transcription of the *Lif* gene. These results suggest that downregulation of the PGR-A isoform at the window of receptivity is necessary for the attenuation of

hedgehog signaling and transcriptional activation of LIF signaling, thereby producing a receptive environment for the attaching conceptus. Although PGR isoforms are critical, they work with other transcription factors to regulate uterine epithelial function. One of these factors is GATA2.

2.2.2. Regulation of PGR signaling by GATA2—GATA binding protein 2 (GATA2) is a transcription factor that has been identified as a PGR target [39] and is a potential modifier of uterine P4 signaling. *Gata2* is expressed in both uterine epithelial and stromal cells and its expression coincides with that of *Pgr* [40]. Mice with germline ablation of *Gata2* display defects in hematopoietic cell development [41], vascular integrity [42], adipocyte differentiation [43], and pituitary function [44]. Mice with uterine ablation of *Gata2* (*Pgr*^{Cre/+}*Gata2*^{flox/flox}) are infertile with defects in conceptus implantation and subsequent uterine stromal decidualization [45]. These mice display stratification in uterine epithelia with increased TRP63 expression, as well as, reduction in PGR target genes and *Pgr* itself. Further cistromic analysis with transcriptomic analysis identified a distinct uterine-specific GATA2 genomic occupancy profile that encompasses most PGR target genes, suggesting a cooperative relationship between the two factors in controlling P4-mediated transcription in the mouse uterus. Importantly, this GATA2-PGR regulatory axis is also observed in endometrial specimens of human subjects consisting of normal and recurrent pregnancy loss patients, which provides a clinical indication and evidence of evolutionary conservation of this pathway [45].

3. Regulation of epithelial-stromal crosstalk

During pregnancy, the increase in P4 signaling initiates a cascade of molecular events within the epithelial and stromal cells of the uterus. This compartmental crosstalk allows the uterine epithelia to stop proliferating, become permissive for proper attachment and implantation of the conceptus, and induces the decidual response in the stromal cells. We and others have mapped out these molecular pathways in this communication between the uterine epithelial and stromal cells as shown in Figure 2. This crosstalk is initiated by the direct regulation of the morphogen Indian hedgehog (IHH) in the uterine epithelial cells by PGR [46, 47]. IHH acts on the uterine stromal cells to regulate the expression of the orphan nuclear receptor Chicken Ovalbumin Upstream Promoter Transcription Factor 2 (COUP-TFII). Downstream of this signaling is the upregulation of the transcription factor neural crest derivative-expressed protein 2 (HAND2). COUP-TFII and HAND2 regulate uterine stromal decidualization, as well as, the inhibition of uterine epithelial cell proliferation. This cascade will be described in the following section.

3.1. IHH

The *Pgr*-initiated epithelial to stromal crosstalk event begins with the induction of IHH in the epithelial cells. IHH is a member of the developmentally regulated morphogens, the Hedgehog (*Hh*) gene family [48]. It has been reported in the regulation of bone development [49, 50], gastrointestinal tract development [51], and embryonic vasculogenesis [52]. It is also expressed in the uterine epithelial cells under the control of the steroid hormone P4 [46, 47]. In humans, IHH decreases in the endometrium of women with endometriosis [53]. In

mice, after ovulation, epithelial PGR regulates the transcription of the *Ihh* gene in the uterine epithelial cells with the maximum production at GD2.5. IHH signals through its receptors Patched 1 (PTCH1) and/or Patched 2 (PTCH2), which are located in uterine stromal cells [46, 47]. Once IHH binds PTCHs, their repression of the membrane signaling protein smoothened (SMO) by PTCHs is removed. SMO then activates a signaling cascade that results in the activation of the glioma-associated oncogene (GLI) transcription factors and the orphan nuclear receptor COUP-TFII [46, 54]. These elements function to prime the stromal cells for decidualization.

Ihh mice exhibit perinatal lethality, presumably caused by respiratory failure due to malformation of the thorax [55]. Mice with uterine ablation of *Ihh* exhibit infertility due to defects in conceptus attachment and stromal cell decidualization [54]. A smoothened (SMO) agonist, administered to these mice results in partial rescue of the decidual phenotype, which demonstrates the importance of the IHH signaling in the induction of decidualization [54]. Furthermore, the generation of a murine model which constitutively activates SMO in the uterus by crossing *Pgr*^{cre/+} mice to *SmoM2⁺ (Gt(ROSA)26Sor^{tm2(Smo/EYFP)Amc})* mice has been generated to better understand the role of IHH signaling in the uterus [56]. Female *Pgr*^{cre/+}*SmoM2⁺* mice are infertile due to an inability of the ova to be fertilized *in vivo* and a failure to undergo the artificially induced decidual response. It also exhibits uterine hypertrophy, and abnormal luminal epithelial cells and a reduction in the number of uterine glands. These findings together indicate a pivotal role of the hedgehog signaling in mediating the epithelial-stromal crosstalk.

3.2. COUP-TFII

COUP-TFII (also known as *Nr2f2*, nuclear receptor subfamily 2, group F, member 2) is expressed within early developing mesenchyme and is vital for epithelial to mesenchymal signaling to promote appropriate development of multiple organs [57, 58]. Ablation of COUP-TFII results in conceptus lethality due to defects in vascularization [59]. Endometrial expression of COUP-TFII is observed primarily in the stromal and vascular cells. Uterine specific ablation of COUP-TFII results in infertility due to failure of attachment and the lack of a decidual response similar to the uterine ablation of *Ihh* mice [60]. Further analyses of the failure of decidualization in mice with uterine ablation of COUP-TFII shows the reduced expression of PGR levels in the endometrial stromal cells along with a decrease in stromal cell proliferation and angiogenesis. In addition to the defects in the uterine stromal cells, uterine epithelial estrogen receptor 1 (ESR1) is elevated and epithelial-specific E2 target genes, such as Mucin 1 (*Muc1*) are up-regulated. The implantation and decidual defects observed in the COUP-TFII mice are rescued by the administration of a low dose of the antiestrogen ICI182780, demonstrating that stromal COUP-TFII is critical for regulating the activity of epithelial ESR1, as well as, uterine epithelial proliferation [61]. These results suggest that COUP-TFII is activated downstream of epithelial PGR and IHH, and promotes the expression of stromal the *Pgr* gene to maintain decidualization. The stromal cells signal to the epithelial cells to inhibit uterine epithelial proliferation and *Esr1* gene targets, such as *Muc1* to prepare uterine epithelial cells for conceptus attachment. Thus, COUP-TFII acts as an epithelial to stromal conduit to govern blastocyst attachment and decidualization by initiating the inhibition of epithelial E2 signaling to allow conceptus attachment and by

promoting the activity of genes critical for preparing the stromal cells for decidualization. In addition to COUP-TFII, heart and neural crest derivatives expressed 2 (HAND2), epidermal growth factor receptor (EGFR), bone morphogenetic protein 2 (BMP2) and Wingless-Type MMTV Integration Site Family, member 4 (WNT4) have been shown to be part of the regulators in uterine stromal cells that regulate epithelial cell proliferation and stromal cell decidualization [62, 63].

3.3. HAND2

The mechanism by which uterine stromal cells regulate proliferation of the uterine epithelial cells and allows P4 to repress uterine proliferation has been uncovered by investigating the role of HAND2 in the mouse uterus. HAND2 was initially identified as necessary for heart development and tissue differentiation through the germline ablation of this gene in mice [64]. Uterine ablation of HAND2 demonstrates that this gene is a critical regulator of the uterine stromal-epithelial communication in response to P4. Loss of the *Hand2* gene in the uterus results in the failure of conceptus attachment and the absence of a decidual response [62]. In addition, the uterine epithelial cells continue to proliferate during the period of receptivity. It was further identified that *Hand2* suppresses the production of several fibroblast growth factors (FGF1, FGF2, FGF9, FGF18) in the stromal cells that act as paracrine mediators of epithelial cell proliferation [62]. Therefore, HAND2 functions to prepare the epithelial cells for conceptus attachment and to promote stromal decidualization during early pregnancy.

4. Uterine stromal cell decidualization

The differentiation of the stromal cells of the uterus from a fibroblastic phenotype to an epithelioid like phenotype is critical to nourish and protect the developing conceptus. In the regulation of mouse stromal cell decidualization during pregnancy, knockout mouse models have been instrumental in identifying the molecular mechanisms that regulate this process. With respect to the IHH-COUP-TFII pathway, epidermal growth factor receptor 1 (ERBB1), BMP2 and WNT4 have been shown to be critical for the regulation of this process.

4.1. ERBB1

Members of the epidermal growth factor (EGF) family, including: amphiregulin (AREG), epidermal growth factor (EGF), transforming growth factor α (TGF- α), and heparin binding epidermal growth factor (HB-EGF) are expressed during the preimplantation period of the uterus, implying the role of EGF signaling in conceptus implantation [65–67]. The expression of HB-EGF, in the luminal epithelial cells surrounding blastocysts at the time of attachment and the effects it has on blastocysts have made it a focus of interest in driving conceptus implantation in the mouse uterus [68, 69]. However, ablation of each of these ligands, either in the whole animal or in the uterus, results in either no effect on fertility or a reduction in fertility, implying functional redundancy in the ligands in regulating uterine function [70, 71]. This may indicate that the receptors for the EGFs, i.e. the ERBBs, may be the limiting factors. There are four members of the ERBB family of receptor tyrosine kinases: the epidermal growth factor receptor (EGFR/ERBB1) and v-erb-b2 erythroblastic leukemia viral oncogene homolog 2–4 (HER2, ERBB3 and ERBB4, respectively). ERBB1–

3 are expressed in the mouse endometrium while ERBB4 localizes predominantly to the muscular myometrium [72–74]. Microarray analysis identifies ERBB1 as downstream of the IHH-COUP-TFII signaling cascade that is required to drive decidualization [75]. *ErbB1–3* genes have been ablated in the mouse uterus with the only member of the ERBB family to affect mouse fertility being ERBB1. These mice have defects in decidualization. Microarray analysis of the *Egfr* uterine knockout showed that ERBB1 is a master regulator of decidualization with BMP2 and WNT4 downstream of EGFR signaling [63].

4.2. BMP2

Bone morphogenetic proteins (BMPs) are multifunctional growth factors that belong to the transforming growth factor β (TGF- β) superfamily. Numerous BMPs are expressed in the uterus during pregnancy in the mouse; yet, only the expression of BMP2, a soluble factor required for osteoblast and osteoclast differentiation [76, 77] is spatiotemporally correlated with implantation [78, 79]. Mice with a universal deletion of the *Bmp2* gene are embryonic lethal due to a failure of proamniotic canal closure in the majority of mice or abnormal cardiac development in the surviving mice [80]. Uterine BMP2 levels are under the control of the major stromal regulator COUP-TFII, implicating a pivotal role of BMP2 in decidualization [60]. As expected, uterine ablation of *Bmp2* results in female mice that are infertile and exhibit a defect in uterine decidualization at the beginning of pregnancy. Nagashima *et al.* generated a conditional deletion of *Bmpr2* (encoded BMP receptor type 2) using *Pgr*-Cre mice, which demonstrates severe hemorrhage at the implantation sites and subsequent fetal growth retardation during midgestation, leading to placental abruption, fetal demise and sterility [81]. Microarray analysis of mice with the conditional ablation of BMP2 in the uterus demonstrate that *Bmp2* regulates decidualization by regulating PGR expression through expression of FK-506-binding proteins (FKBPs), which stabilizes and promotes the activity of PGR [82]. Additionally, BMP2 regulates a multitude of targets in the stromal cells, including prostaglandin-endoperoxide synthase 1 (PTGS1) and the noncanonical WNT ligands, WNT4 and WNT6.

4.3. WNT4

Wnt genes encode secreted glycoproteins that are homologous to the *Drosophila* segment polarity gene *wingless* (*wg*) and regulate stem cell fate, cell differentiation, and tissue growth [83] via canonical and non-canonical signaling pathways [84]. The canonical WNT signaling pathway involves binding of frizzled (FZD) receptors and inhibition of β -catenin (encoded by *Ctnnb1* gene) degradation, resulting in nuclear translocation and activation of target genes. The non-canonical WNT signaling encompasses a variety of signaling pathways that involve different WNT receptors (i.e. ROR2 and RYK) and mediators (i.e. calcium, MAPK8/9/10, and CAMK2) that regulate cell migration and movement [85]. A subset of WNTs (WNT4, WNT5A, and WNT7A) is involved in müllerian duct patterning and differentiation during development of the female reproductive tract in the embryo [86]. Thus, analysis of the *in vivo* role of these genes in adult uterine function has been limited [87, 88]. Conditional ablation of *Wnt4* in the uterus results in female sterility. These mice display alterations in the differentiation of the uterine luminal epithelial cells, a reduction in the number of uterine glands, as well as, failure in decidualization, thereby, exhibiting subfertility [89]. Like *Bmp2*, *Wnt4* regulates the responsiveness of uterine stromal cells to

P4, suggesting that WNT4 signaling is also required for optimal PGR signaling. Notably, WNT4 gene expression is under direct regulation of COUP-TFII, as indicated by transcriptome and COUP-TFII binding profiles in human endometrial stromal cells [90].

5. Conclusions and future directions

In all mammals, successful establishment and maintenance of pregnancy requires complex remodeling of the endometrium to orchestrate implantation, which includes alteration in gene expressions that coordinate with conceptus attachment to uterine epithelial cells (domestic animals), invasion to uterine stromal cells (rodents and primates) and then, cause phenotypic modification of stromal cells, silencing of receptors for P4 and E2, and suppression of genes for immune recognition, as well as, enhancing signals for pregnancy recognition. Advancements in technology and the use of genetically engineered mouse models have extensively enhanced our understanding of these timely orchestrated molecular events that are critical for pregnancy and P4-dependency. In particular, cistronic, transcriptomic coupled with proteomic tools helped identify multiple novel PGR targets, followed by the generation of uterine compartment-specific KO mouse models to discover their roles as either downstream effectors or coregulators involved in important uterine function during the window of receptivity. Recently, new gene editing technology, such as CRISPR/Cas9 system has opened a new era for the generation of transgenic animal models in a more efficient and economical way that can help further identify uterine-specific enhancer or promoter regions of these PGR-associated genes. For example, PGR and GATA2 both occupy at putative enhancers of the *Sox17* and *Ihh* genes [45] that have pivotal uterine functions [54, 91, 92]. While results from *in vitro* assays suggest a cooperation of PGR and GATA2 in the regulation of enhancers for the *Sox17* and *Ihh* genes, the *in vivo* function of such enhancers remains to be demonstrated, perhaps by CRISPR technology. Furthermore, advanced statistical and bioinformatics tools are available for integrated analysis of genome-wide data from both human subjects and mouse models, which can help to identify genetic networks and regulatory mechanisms that are conserved across species [45, 93–95]. Importantly, mapping out evolutionary conservation and difference on regulatory pathways would help better interpretation on data generated from preclinical investigations. In summary, the PGR exerts pleiotropic effects by regulating multiple types of proteins including ligands, receptors, signaling proteins, and transcription factors between uterine epithelial and stromal cells. Understanding these mechanisms is not only necessary for fertility improvement, but also the development of new targeted therapies for its associated disease like endometriosis and endometrial cancer.

Acknowledgments

We thank Janet L. DeMayo for manuscript editing. This work was supported by the Intramural Research Program of the National Institute of Environmental Health Sciences: Project Z1AES103311-01 (FJD).

References

1. Chandra A, Copen CE, Stephen EH. Infertility and impaired fecundity in the United States, 1982–2010: data from the National Survey of Family Growth. *Natl Health Stat Report*. 2013;1–18. 11 p following 19.

2. La Sala GB, Nucera G, Gallinelli A, Nicoli A, Villani MT, Blickstein I. Spontaneous embryonic loss after in vitro fertilization with and without intracytoplasmic sperm injection. *Fertil Steril*. 2004; 82:1536–1539. [PubMed: 15589856]
3. Adams NR, DeMayo FJ. The Role of Steroid Hormone Receptors in the Establishment of Pregnancy in Rodents. *Adv Anat Embryol Cell Biol*. 2015; 216:27–49. [PubMed: 26450493]
4. Lee KY, DeMayo FJ. Animal models of implantation. *Reproduction*. 2004; 128:679–695. [PubMed: 15579585]
5. Wang X, Frank JW, Little DR, Dunlap KA, Satterfield MC, Burghardt RC, Hansen TR, Wu G, Bazer FW. Functional role of arginine during the peri-implantation period of pregnancy. I. Consequences of loss of function of arginine transporter SLC7A1 mRNA in ovine conceptus trophoctoderm. *FASEB J*. 2014; 28:2852–2863. [PubMed: 24627544]
6. Paria BC, Lim H, Das SK, Reese J, Dey SK. Molecular signaling in uterine receptivity for implantation. *Semin Cell Dev Biol*. 2000; 11:67–76. [PubMed: 10873704]
7. Cha J, Sun X, Dey SK. Mechanisms of implantation: strategies for successful pregnancy. *Nat Med*. 2012; 18:1754–1767. [PubMed: 23223073]
8. Ma WG, Song H, Das SK, Paria BC, Dey SK. Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation. *Proc Natl Acad Sci U S A*. 2003; 100:2963–2968. [PubMed: 12601161]
9. Vasquez YM, DeMayo FJ. Role of nuclear receptors in blastocyst implantation. *Semin Cell Dev Biol*. 2013; 24:724–735. [PubMed: 23994285]
10. Large MJ, DeMayo FJ. The regulation of embryo implantation and endometrial decidualization by progesterone receptor signaling. *Mol Cell Endocrinol*. 2012; 358:155–165. [PubMed: 21821095]
11. Franco HL, Jeong JW, Tsai SY, Lydon JP, DeMayo FJ. In vivo analysis of progesterone receptor action in the uterus during embryo implantation. *Semin Cell Dev Biol*. 2008; 19:178–186. [PubMed: 18280760]
12. Rubel CA, Jeong JW, Tsai SY, Lydon JP, Demayo FJ. Epithelial-stromal interaction and progesterone receptors in the mouse uterus. *Semin Reprod Med*. 2010; 28:27–35. [PubMed: 20104426]
13. Winuthayanon W, Hewitt SC, Orvis GD, Behringer RR, Korach KS. Uterine epithelial estrogen receptor alpha is dispensable for proliferation but essential for complete biological and biochemical responses. *Proc Natl Acad Sci U S A*. 2010; 107:19272–19277. [PubMed: 20974921]
14. Wetendorf M, Wu SP, Wang X, Creighton CJ, Wang T, Lanz RB, Blok L, Tsai SY, Tsai MJ, Lydon JP, DeMayo FJ. Decreased epithelial progesterone receptor A at the window of receptivity is required for preparation of the endometrium for embryo attachment. *Biol Reprod*. 2017; 96:313–326. [PubMed: 28203817]
15. Bhurke AS, Bagchi IC, Bagchi MK. Progesterone-Regulated Endometrial Factors Controlling Implantation. *Am J Reprod Immunol*. 2016; 75:237–245. [PubMed: 26804062]
16. Pawar S, Hantak AM, Bagchi IC, Bagchi MK. Minireview: Steroid-regulated paracrine mechanisms controlling implantation. *Mol Endocrinol*. 2014; 28:1408–1422. [PubMed: 25051170]
17. Hantak AM, Bagchi IC, Bagchi MK. Role of uterine stromal-epithelial crosstalk in embryo implantation. *Int J Dev Biol*. 2014; 58:139–146. [PubMed: 25023679]
18. Ramathal CY, Bagchi IC, Taylor RN, Bagchi MK. Endometrial decidualization: of mice and men. *Semin Reprod Med*. 2010; 28:17–26. [PubMed: 20104425]
19. Bagchi IC, Cheon YP, Li Q, Bagchi MK. Progesterone receptor-regulated gene networks in implantation. *Front Biosci*. 2003; 8:s852–861. [PubMed: 12957875]
20. Wang H, Dey SK. Roadmap to embryo implantation: clues from mouse models. *Nat Rev Genet*. 2006; 7:185–199. [PubMed: 16485018]
21. Spencer TE, Johnson GA, Bazer FW, Burghardt RC. Implantation mechanisms: insights from the sheep. *Reproduction*. 2004; 128:657–668. [PubMed: 15579583]
22. Cha J, Dey SK. Cadence of procreation: orchestrating embryo-uterine interactions. *Semin Cell Dev Biol*. 2014; 34:56–64. [PubMed: 24862857]
23. Groothuis PG, Dassen HH, Romano A, Punyadeera C. Estrogen and the endometrium: lessons learned from gene expression profiling in rodents and human. *Hum Reprod Update*. 2007; 13:405–417. [PubMed: 17584823]

24. Abrahamsohn PA, Zorn TM. Implantation and decidualization in rodents. *J Exp Zool.* 1993; 266:603–628. [PubMed: 8371101]
25. Finn CA, Martin L. The control of implantation. *J Reprod Fertil.* 1974; 39:195–206. [PubMed: 4605229]
26. McCormack JT, Greenwald GS. Evidence for a preimplantation rise in oestradiol-17beta levels on day 4 of pregnancy in the mouse. *Journal of reproduction and fertility.* 1974; 41:297–301. [PubMed: 4452972]
27. Lessey BA. Assessment of endometrial receptivity. *Fertil Steril.* 2011; 96:522–529. [PubMed: 21880273]
28. Tan J, Paria BC, Dey SK, Das SK. Differential uterine expression of estrogen and progesterone receptors correlates with uterine preparation for implantation and decidualization in the mouse. *Endocrinology.* 1999; 140:5310–5321. [PubMed: 10537162]
29. Couse JF, Lindzey J, Grandien K, Gustafsson JA, Korach KS. Tissue distribution and quantitative analysis of estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERbeta) messenger ribonucleic acid in the wild-type and ERalpha-knockout mouse. *Endocrinology.* 1997; 138:4613–4621. [PubMed: 9348186]
30. Curtis Hewitt S, Goulding EH, Eddy EM, Korach KS. Studies using the estrogen receptor alpha knockout uterus demonstrate that implantation but not decidualization-associated signaling is estrogen dependent. *Biol Reprod.* 2002; 67:1268–1277. [PubMed: 12297545]
31. Chen JR, Cheng JG, Shatzer T, Sewell L, Hernandez L, Stewart CL. Leukemia inhibitory factor can substitute for nidatory estrogen and is essential to inducing a receptive uterus for implantation but is not essential for subsequent embryogenesis. *Endocrinology.* 2000; 141:4365–4372. [PubMed: 11108244]
32. Cheng JG, Rodriguez CI, Stewart CL. Control of uterine receptivity and embryo implantation by steroid hormone regulation of LIF production and LIF receptor activity: towards a molecular understanding of "the window of implantation". *Reviews in endocrine & metabolic disorders.* 2002; 3:119–126. [PubMed: 12007288]
33. Charnock-Jones DS, Sharkey AM, Fenwick P, Smith SK. Leukaemia inhibitory factor mRNA concentration peaks in human endometrium at the time of implantation and the blastocyst contains mRNA for the receptor at this time. *Journal of reproduction and fertility.* 1994; 101:421–426. [PubMed: 7932378]
34. Cooke PS, Buchanan DL, Young P, Setiawan T, Brody J, Korach KS, Taylor J, Lubahn DB, Cunha GR. Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium. *Proc Natl Acad Sci U S A.* 1997; 94:6535–6540. [PubMed: 9177253]
35. Li X, O'Malley BW. Unfolding the action of progesterone receptors. *J Biol Chem.* 2003; 278:39261–39264. [PubMed: 12893816]
36. Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA Jr, Shyamala G, Conneely OM, O'Malley BW. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev.* 1995; 9:2266–2278. [PubMed: 7557380]
37. Mulac-Jericevic B, Mullinax RA, DeMayo FJ, Lydon JP, Conneely OM. Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform. *Science.* 2000; 289:1751–1754. [PubMed: 10976068]
38. Mulac-Jericevic B, Lydon JP, DeMayo FJ, Conneely OM. Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. *Proc Natl Acad Sci U S A.* 2003; 100:9744–9749. [PubMed: 12897242]
39. 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. [PubMed: 22638070]
40. Rubel CA, Franco HL, Jeong JW, Lydon JP, DeMayo FJ. GATA2 is expressed at critical times in the mouse uterus during pregnancy. *Gene Expr Patterns.* 2012; 12:196–203. [PubMed: 22476030]
41. Tsai FY, Keller G, Kuo FC, Weiss M, Chen J, Rosenblatt M, Alt FW, Orkin SH. An early haematopoietic defect in mice lacking the transcription factor GATA-2. *Nature.* 1994; 371:221–226. [PubMed: 8078582]

42. Johnson KD, Hsu AP, Ryu MJ, Wang J, Gao X, Boyer ME, Liu Y, Lee Y, Calvo KR, Keles S, Zhang J, Holland SM, Bresnick EH. Cis-element mutated in GATA2-dependent immunodeficiency governs hematopoiesis and vascular integrity. *J Clin Invest.* 2012; 122:3692–3704. [PubMed: 22996659]
43. Tong Q, Dalgin G, Xu H, Ting CN, Leiden JM, Hotamisligil GS. Function of GATA transcription factors in preadipocyte-adipocyte transition. *Science.* 2000; 290:134–138. [PubMed: 11021798]
44. Charles MA, Saunders TL, Wood WM, Owens K, Parlow AF, Camper SA, Ridgway EC, Gordon DF. Pituitary-specific Gata2 knockout: effects on gonadotrope and thyrotrope function. *Mol Endocrinol.* 2006; 20:1366–1377. [PubMed: 16543408]
45. Rubel CA, Wu SP, Lin L, Wang T, Lanz RB, Li X, Kommagani R, Franco HL, Camper SA, Tong Q, Jeong JW, Lydon JP, DeMayo FJ. A Gata2-Dependent Transcription Network Regulates Uterine Progesterone Responsiveness and Endometrial Function. *Cell Rep.* 2016; 17:1414–1425. [PubMed: 27783953]
46. Takamoto N, Zhao B, Tsai SY, DeMayo FJ. Identification of Indian hedgehog as a progesterone-responsive gene in the murine uterus. *Mol Endocrinol.* 2002; 16:2338–2348. [PubMed: 12351698]
47. Matsumoto H, Zhao X, Das SK, Hogan BL, Dey SK. Indian hedgehog as a progesterone-responsive factor mediating epithelial-mesenchymal interactions in the mouse uterus. *Dev Biol.* 2002; 245:280–290. [PubMed: 11977981]
48. Ingham PW, McMahon AP. Hedgehog signaling in animal development: paradigms and principles. *Genes Dev.* 2001; 15:3059–3087. [PubMed: 11731473]
49. Pathi S, Rutenberg JB, Johnson RL, Vortkamp A. Interaction of Ihh and BMP/Noggin signaling during cartilage differentiation. *Dev Biol.* 1999; 209:239–253. [PubMed: 10328918]
50. Vortkamp A. The Indian hedgehog--PTHrP system in bone development. *Ernst Schering Res Found Workshop.* 2000:191–209. [PubMed: 10943311]
51. Ramalho-Santos M, Melton DA, McMahon AP. Hedgehog signals regulate multiple aspects of gastrointestinal development. *Development.* 2000; 127:2763–2772. [PubMed: 10821773]
52. Dyer MA, Farrington SM, Mohn D, Munday JR, Baron MH. Indian hedgehog activates hematopoiesis and vasculogenesis and can respecify prospective neuroectodermal cell fate in the mouse embryo. *Development.* 2001; 128:1717–1730. [PubMed: 11311154]
53. Smith K, Alnifaity R, Wei Q, Nieman LK. Endometrial Indian hedgehog expression is decreased in women with endometriosis. *Fertil Steril.* 2011; 95:2738–2741. e2731–2733. [PubMed: 21640343]
54. 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. [PubMed: 16951680]
55. St-Jacques B, Hammerschmidt M, McMahon AP. Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. *Genes Dev.* 1999; 13:2072–2086. [PubMed: 10465785]
56. 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. [PubMed: 20130264]
57. Pereira FA, Qiu Y, Tsai MJ, Tsai SY. Chicken ovalbumin upstream promoter transcription factor (COUP-TF): expression during mouse embryogenesis. *J Steroid Biochem Mol Biol.* 1995; 53:503–508. [PubMed: 7626501]
58. Tsai SY, Tsai MJ. Chick ovalbumin upstream promoter-transcription factors (COUP-TFs): coming of age. *Endocr Rev.* 1997; 18:229–240. [PubMed: 9101138]
59. Pereira FA, Qiu Y, Zhou G, Tsai MJ, Tsai SY. The orphan nuclear receptor COUP-TFII is required for angiogenesis and heart development. *Genes Dev.* 1999; 13:1037–1049. [PubMed: 10215630]
60. 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. [PubMed: 17590085]
61. Lee DK, Kurihara I, Jeong JW, Lydon JP, DeMayo FJ, Tsai MJ, Tsai SY. Suppression of ERalpha activity by COUP-TFII is essential for successful implantation and decidualization. *Mol Endocrinol.* 2010; 24:930–940. [PubMed: 20219888]

62. 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. [PubMed: 21330545]
63. Large MJ, Wetendorf M, Lanz RB, Hartig SM, Creighton CJ, Mancini MA, Kovanci E, Lee KF, Threadgill DW, Lydon JP, Jeong JW, DeMayo FJ. The epidermal growth factor receptor critically regulates endometrial function during early pregnancy. *PLoS Genet*. 2014; 10:e1004451. [PubMed: 24945252]
64. Srivastava D, Thomas T, Lin Q, Kirby ML, Brown D, Olson EN. Regulation of cardiac mesodermal and neural crest development by the bHLH transcription factor, dHAND. *Nat Genet*. 1997; 16:154–160. [PubMed: 9171826]
65. Das SK, Chakraborty I, Paria BC, Wang XN, Plowman G, Dey SK. Amphiregulin is an implantation-specific and progesterone-regulated gene in the mouse uterus. *Mol Endocrinol*. 1995; 9:691–705. [PubMed: 8592515]
66. Das SK, Das N, Wang J, Lim H, Schryver B, Plowman GD, Dey SK. Expression of betacellulin and epiregulin genes in the mouse uterus temporally by the blastocyst solely at the site of its apposition is coincident with the "window" of implantation. *Dev Biol*. 1997; 190:178–190. [PubMed: 9344537]
67. Tamada H, Das SK, Andrews GK, Dey SK. Cell-type-specific expression of transforming growth factor- α in the mouse uterus during the peri-implantation period. *Biol Reprod*. 1991; 45:365–372. [PubMed: 1686188]
68. Das SK, Wang XN, Paria BC, Damm D, Abraham JA, Klagsbrun M, Andrews GK, Dey SK. Heparin-binding EGF-like growth factor gene is induced in the mouse uterus temporally by the blastocyst solely at the site of its apposition: a possible ligand for interaction with blastocyst EGF-receptor in implantation. *Development*. 1994; 120:1071–1083. [PubMed: 8026321]
69. Raab G, Kover K, Paria BC, Dey SK, Ezzell RM, Klagsbrun M. Mouse preimplantation blastocysts adhere to cells expressing the transmembrane form of heparin-binding EGF-like growth factor. *Development*. 1996; 122:637–645. [PubMed: 8625815]
70. Luetke NC, Qiu TH, Fenton SE, Troyer KL, Riedel RF, Chang A, Lee DC. Targeted inactivation of the EGF and amphiregulin genes reveals distinct roles for EGF receptor ligands in mouse mammary gland development. *Development*. 1999; 126:2739–2750. [PubMed: 10331984]
71. 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 U S A*. 2007; 104:18315–18320. [PubMed: 17986609]
72. Das SK, Tsukamura H, Paria BC, Andrews GK, Dey SK. Differential expression of epidermal growth factor receptor (EGF-R) gene and regulation of EGF-R bioactivity by progesterone and estrogen in the adult mouse uterus. *Endocrinology*. 1994; 134:971–981. [PubMed: 7507841]
73. Lim H, Das SK, Dey SK. erbB genes in the mouse uterus: cell-specific signaling by epidermal growth factor (EGF) family of growth factors during implantation. *Dev Biol*. 1998; 204:97–110. [PubMed: 9851845]
74. Lim H, Dey SK, Das SK. Differential expression of the erbB2 gene in the periimplantation mouse uterus: potential mediator of signaling by epidermal growth factor-like growth factors. *Endocrinology*. 1997; 138:1328–1337. [PubMed: 9048643]
75. Franco HL, Lee KY, Broadus RR, White LD, Lanske B, Lydon JP, Jeong JW, DeMayo FJ. Ablation of Indian hedgehog in the murine uterus results in decreased cell cycle progression, aberrant epidermal growth factor signaling, and increased estrogen signaling. *Biol Reprod*. 2010; 82:783–790. [PubMed: 20056671]
76. Canalis E, Economides AN, Gazzerro E. Bone morphogenetic proteins, their antagonists, and the skeleton. *Endocr Rev*. 2003; 24:218–235. [PubMed: 12700180]
77. Wang EA, Rosen V, D'Alessandro JS, Bauduy M, Cordes P, Harada T, Israel DI, Hewick RM, Kerns KM, LaPan P, et al. Recombinant human bone morphogenetic protein induces bone formation. *Proc Natl Acad Sci U S A*. 1990; 87:2220–2224. [PubMed: 2315314]
78. 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 U S A*. 2001; 98:1047–1052. [PubMed: 11158592]

79. Ying Y, Zhao GQ. Detection of multiple bone morphogenetic protein messenger ribonucleic acids and their signal transducer, Smad1, during mouse decidualization. *Biol Reprod.* 2000; 63:1781–1786. [PubMed: 11090449]
80. Zhang H, Bradley A. Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. *Development.* 1996; 122:2977–2986. [PubMed: 8898212]
81. 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. [PubMed: 23676498]
82. 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. [PubMed: 17515606]
83. Polakis P. Wnt signaling and cancer. *Genes Dev.* 2000; 14:1837–1851. [PubMed: 10921899]
84. Angers S, Moon RT. Proximal events in Wnt signal transduction. *Nat Rev Mol Cell Biol.* 2009; 10:468–477. [PubMed: 19536106]
85. van Amerongen R. Alternative Wnt pathways and receptors. *Cold Spring Harb Perspect Biol.* 2012; 4
86. Mullen RD, Behringer RR. Molecular genetics of Mullerian duct formation, regression and differentiation. *Sex Dev.* 2014; 8:281–296. [PubMed: 25033758]
87. Vainio S, Heikkila M, Kispert A, Chin N, McMahon AP. Female development in mammals is regulated by Wnt-4 signalling. *Nature.* 1999; 397:405–409. [PubMed: 9989404]
88. Mericskay M, Kitajewski J, Sassoon D. Wnt5a is required for proper epithelial-mesenchymal interactions in the uterus. *Development.* 2004; 131:2061–2072. [PubMed: 15073149]
89. 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. [PubMed: 21163860]
90. 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. [PubMed: 24176914]
91. Guimaraes-Young A, Neff T, Dupuy AJ, Goodheart MJ. Conditional deletion of Sox17 reveals complex effects on uterine adenogenesis and function. *Dev Biol.* 2016; 414:219–227. [PubMed: 27102016]
92. Hirate Y, Suzuki H, Kawasumi M, Takase HM, Igarashi H, Naquet P, Kanai Y, Kanai-Azuma M. Mouse Sox17 haploinsufficiency leads to female subfertility due to impaired implantation. *Sci Rep.* 2016; 6:24171. [PubMed: 27053385]
93. Qin J, Wu SP, Creighton CJ, Dai F, Xie X, Cheng CM, Frolov A, Ayala G, Lin X, Feng XH, Ittmann MM, Tsai SJ, Tsai MJ, Tsai SY. COUP-TFII inhibits TGF-beta-induced growth barrier to promote prostate tumorigenesis. *Nature.* 2013; 493:236–240. [PubMed: 23201680]
94. Qin J, Lee HJ, Wu SP, Lin SC, Lanz RB, Creighton CJ, DeMayo FJ, Tsai SY, Tsai MJ. Androgen deprivation-induced NCoA2 promotes metastatic and castration-resistant prostate cancer. *J Clin Invest.* 2014; 124:5013–5026. [PubMed: 25295534]
95. Wu SP, Kao CY, Wang L, Creighton CJ, Yang J, Donti TR, Harmancey R, Vasquez HG, Graham BH, Bellen HJ, Taegtmeier H, Chang CP, Tsai MJ, Tsai SY. Increased COUP-TFII expression in adult hearts induces mitochondrial dysfunction resulting in heart failure. *Nat Commun.* 2015; 6:8245. [PubMed: 26356605]

Fig. 1.

Schematic diagram of the window of receptivity governed by ovarian steroid progesterone (P_4) for uterine epithelial-stromal communication. PGR regulates growth factors that prime the stromal cells for decidualization, while stromal cells produce factors that control epithelial cell proliferation. The crosstalk thereby allows conceptus attachment, invasion as well as establishment and maintenance of pregnancy. LE, luminal epithelium.

Fig. 2.

Epithelial PGR regulates multiple signaling pathways in the endometrium and transduce signals via uterine epithelial-stromal interactions. Epithelial PGR regulates transcription of *Ihh*, which acts on stromal cells via its receptors, PTCH1 and PTCH2, as well as its downstream mediator COUP-TFII and stromal PGR. This pathway primes stromal cells for decidualization via BMP2 and WNT4. Induction of BMP2 and WNT4 also requires EGFR signaling. In addition, HAND2 expression is promoted directly by stromal PGR and COUP-TFII. HAND2 then inhibits production of FGF ligands, thereby stop proliferative signaling to the uterine epithelial cells. PGR, progesterone receptor; GATA2, GATA binding protein 2;

IHH, indian hedgehog; PTCH1, patched 1; PTCH2, patched 2; COUP-TFII, chicken ovalbumin upstream transcription factor II; BMP2, bone morphogen protein 2; WNT4, Wnt family member 4; EGFR, epidermal growth factor receptor; HAND2, heart- and neural crest derivative-expressed protein 2; FGF, fibroblast growth factor.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript