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PROGESTERONE ACTION IN THE HUMAN ENDOMETRIUM: INDUCTION OF A UNIQUE TISSUE ENVIRONMENT WHICH LIMITS MATRIX METALLOPROTEINASE (MMP) EXPRESSION

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Progesterone and the Menstruation Response
4. Progesterone and Endometrial Maturation
5. TGF-beta and Endometrial Maturation
6. Cooperative Interactions of Progesterone, Retinoic Acid and TGF-beta
7. Perspectives
8. Acknowledgements
9. References

1. ABSTRACT

The endometrium is a unique adult tissue which, in the absence of pregnancy or disease, undergoes cyclic breakdown and regrowth approximately 400 times during a woman's reproductive life. The chances of reproductive success during each cycle depends on appropriate, cell-specific responses to steroids, including expression of matrix metalloproteinases (MMPs). Normal endometrial MMP regulation in response to either estrogen or progesterone requires additional, cell-specific interactions mediated by various growth factors and cytokines. During endometrial maturation, progesterone, retinoic acid and TGF-beta act cooperatively, providing a remarkable biological balance to regulate expression of MMPs in the highly steroid-sensitive endometrium. Exploring the regulatory actions of locally produced growth factors and cytokines on members of the MMP family and their inhibitors will allow a better understanding of the unique physiology of the human endometrium under the influence of progesterone.

2. INTRODUCTION

In some mammalian species, environmental factors, such as seasonal changes, light cycles, or the presence or absence of a male, can have a dramatic influence over the female reproductive cycle. In humans, nutritional and stress-related changes in the menstrual cycle are often associated with periods of infertility, while most other external, environmental elements of control over reproductive processes have been lost along our evolutionary pathway. Under conditions of normal health and nutrition, the cyclic responses of the human endometrium to changing patterns of ovarian steroids has become the most important aspect of reproductive success and thus the survival of our species. The development of endometrial biology among humans and primates provides an appropriately secure site for maternal-fetal exchange, initially acting in concert with ovarian function while subsequently meeting the demands of live birth. In adults, the endometrium can be viewed as three morphologically and functionally distinct layers: the *stratum basalis* which lies adjacent to the myometrium, the *stratum spongiosum* or intermediate layer and the *stratum compactum*, the uppermost region. It is from the *stratum basalis* that the endometrium regenerates after each menstrual shedding, while the two uppermost layers, referred to jointly as the *stratum functionalis*, undergo coordinated histologic and cytologic changes throughout the menstrual cycle (1). The restructuring of vascular, stromal and glandular elements, derived from newly growing cells from the *stratum basalis*, essentially reconstructs the functional components of the adult endometrium, a process which is biologically similar to developmental processes during organ formation in the embryo. Across each menstrual cycle, steroids drive extensive tissue turnover in the endometrium, including tissue sloughing, tissue repair and a high degree of co-ordinated cell proliferation and differentiation in preparation for pregnancy. As is true during embryonic development, endometrial remodeling requires the cooperative actions of numerous MMPs, a family of secreted, locally active enzymes which are involved in multiple processes required for reproductive success (2).

Although the action of MMPs is intimately linked to normal endometrial biology, the inappropriate expression of these enzymes is equally linked to the disruption of tissue integrity and function associated with a number of uterine pathologies. To date, altered MMP expression has been identified among patients with dysfunctional uterine bleeding (3, 4), early pregnancy loss (5), endometriosis (6-8) and cancer (9). Obviously, the cellular and molecular mechanisms which act to limit the expression and

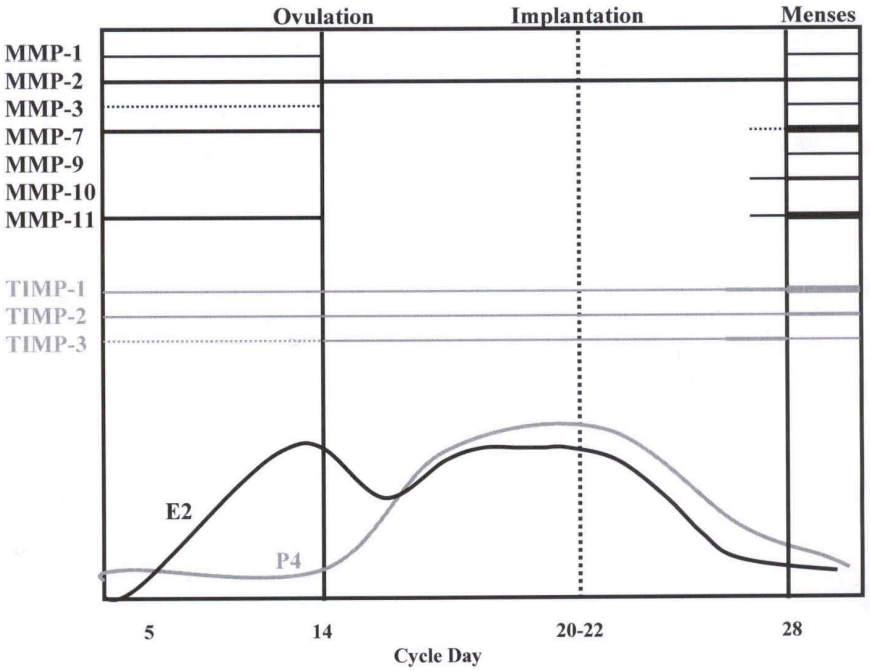

Figure 1. Summary of the expression pattern of selected MMPs and TIMPs during the menstrual cycle. Intensity of expression is indicated by thickness of bar. Relative levels of estradiol (E2) and progesterone (P4) are also indicated.

action of these enzymes play a critical role during each normal menstrual cycle. As opposed to non-reproductive tissues, transcriptional regulation of endometrial MMP expression appears to be significantly impacted by ovarian steroids, acting in concert with local mediators, including growth factors, members of the retinoid family and pro-inflammatory cytokines (10). Additionally, tissue inhibitors of MMPs (TIMPs), plasminogen activator inhibitor (PAI-1), and general protease inhibitors such as a-2 macroglobulin and a-1 antitrypsin also can impact the expression and activity of these enzymes throughout the reproductive cycle (2). Importantly, the expression and action of locally produced factors reflects the changing influence of steroid exposure on endometrial physiology, leading to focal, cell-specific patterns of MMP expression. Under the influence of progesterone, the endometrium initiates a series of unique cellular differentiation and functional activities which prepare the uterus for establishment and maintenance of pregnancy (2). In this review, we will explore progesterone action in the human endometrium, focusing on the role of this steroid in mediating broad suppression of the MMP family within the environment of early pregnancy. We will also discuss the role of cell-cell communication as a component of MMP suppression, focusing on the role of transforming growth factor-beta (TGF-beta) as a key progesterone-mediated paracrine factor. Lastly, we will discuss the emerging role of local retinoic acid synthesis during stromal decidualization, as a potential regulator of both TGF-beta and MMP expression at the maternal-fetal interface. Since menstruation marks both the failure to establish pregnancy and the beginning of a new menstrual cycle, we will begin our discussion of progesterone action with a consideration of MMP expression during menstruation-related events.

3. PROGESTERONE AND THE MENSTRUATION RESPONSE

During a woman's reproductive life, changing patterns of ovarian estradiol and progesterone production direct a predictable pattern of endometrial growth and maturation which temporally connects the endometrium to ooctye maturation, ovulation, fertilization, embryo transport and implantation. In response to these steroids, the endometrium develops extensive surface and glandular epithelium with a rich supporting stroma consisting of pluripotent mesenchymal cells, vascular elements and a diverse, cycle-specific population of invasive cells of hemopoietic origin. Although the process of menstruation is generally recognized as the beginning of a new menstrual cycle, the loss of steroid support to the endometrium is also a reflection of the failure to successfully reproduce. Numerous theories have been proposed to explain the temporal events which initiate menstruation based on both vascular and non-vascular tissue changes; nevertheless, a number of recent studies have indicated that MMPs may play an important and perhaps primary role during menstruation (11-13). As shown in figure 1, the expression of MMPs is lowest during the early to mid-secretory phase of the cycle, corresponding to the time of maximum progesterone exposure, while the expression of multiple MMPs subsequently increases to their highest level during the immediate premenstrual and menstrual phases as progesterone support falls (11, 12, 14-16).

Although the regulation of specific MMPs and TIMPs during menstruation likely involves complex molecular mechanisms, falling progesterone levels clearly triggers broad changes in MMP and TIMP expression

across the upper region of the human endometrium (11, 12). One of the earliest cellular changes noted in the endometrium in response to steroid withdrawal at the end of the cycle is the focal expression of MMP-7 among isolated clusters of glandular epithelium (12). As ovarian steroids decline, the local action of tumor necrosis factor-alpha (TNF-alpha) has been proposed as a key mediator of the induction of apoptosis in endometrial epithelium (17), while IL1-alpha and IL1-beta have been suggested to be involved in the stimulation of MMP expression during menstruation (16, 18-20). The local production of pro-inflammatory cytokines, predominantly by epithelial cells (21, 22), may subsequently initiate focal increases in MMP expression among other cell-types within the *stratum functionalis* (18). In response to this inflammatory-like environment, increased expression of multiple MMPs, as well as TIMPs, have been observed in the human and primate endometrium in association with menstrual breakdown and bleeding (11, 23, 24). Taken together, these studies suggest that the increased expression of members of the MMP family in response to decreased steroid support is largely associated with the increased activity of pro-inflammatory cytokines (16, 18-20). However, an inflammatory-like environment also exists at the maternal-fetal interface, suggesting that continued ovarian progesterone production during early pregnancy maintains endometrial tissue stability by controlling the balance of MMP and TIMP expression.

The virtual absence of expression of most MMPs during the secretory phase of the menstrual cycle suggests that these enzymes are not part of endometrial preparation for implantation and placentation. In contrast, while the MMPs are largely suppressed by progesterone, the expression of TIMP-1 and TIMP-3 appears to rise slowly across the secretory phase, prior to menstruation (11, 25, 26). Additionally, the secretion of TIMP-1 and TIMP-3 by isolated endometrial stromal cells increases *in vitro* during progesterone-induced decidualization (25, 27). Although the local tissue environment of normal human pregnancy is difficult to study for ethical reasons, elevated expression of MMP-7 has been detected among epithelial cells at sites of ectopic tubal pregnancy in contrast to little expression of this MMP within the eutopic endometrial epithelium of these patients (28). Using an *in vitro* system to model the environment of pregnancy, we find that progesterone largely prevents the stimulation of stromal-specific MMP-3 by numerous pro-inflammatory cytokines (29, 30). Together, these observations strongly suggest that a key element of progesterone action within the fundus of the endometrium is the creation of a unique tissue environment capable of limiting the expression and action of MMPs within the inflammatory-like conditions of early pregnancy.

4. PROGESTERONE AND ENDOMETRIAL MATURATION

As the endometrium prepares for pregnancy, an appropriate balance of estrogen and progesterone exposure appears to be important for cell-specific MMP regulation since the expression of these enzymes increases dramatically upon withdrawal of these steroids either *in vivo* or *in vitro* (14, 29, 31). Therefore, the predictable and precisely controlled regulation of MMPs during the menstrual cycle requires not only an appropriate balance of ovarian steroids, but also the expression of specific isotypes of steroid receptors among responsive endometrial cell types. Using classical biochemical analysis, Lessey et al., (32) demonstrated that the highest levels of total tissue estrogen receptor (ER) is detected during the late proliferative to early secretory transition while progesterone receptor (PR) levels peak later during the early to mid-secretory stage of the menstrual cycle. The PR occurs as two distinct isoforms, PR-A and PR-B, which are derived from a single gene as a consequence of alternate initiation of transcription (33). The PR-B isoform appears to mediate most endometrial responses to progesterone (34) and alterations in the ratio of PR isotypes may contribute to the pathophysiology of endometrial disease. For example, an elevated expression of PR-A is associated with endometriosis (35), a disease which exhibits a diminished *in vivo* and *in vitro* response to progesterone-mediated MMP suppression (8). Recent studies in our laboratory have focused on identifying the direct and indirect mechanisms by which progesterone acts to maintain control over endometrial MMP expression within the inflammatory-like environment of early pregnancy.

As noted above, changing levels of ovarian steroid support has a dramatic influence over the focal, cell-type specific expression of multiple MMP and TIMP genes across the menstrual cycle and during early pregnancy. During endometrial growth, cell-specific expression of MMPs occurs in focal areas of tissue remodeling, predominately regulated by estrogen-mediated paracrine factors (36,37,38). In contrast to the expression of endometrial MMPs under the influence of estrogen, the majority of these enzymes are not detected by *in situ* hybridization following progesterone exposure during the early to mid-secretory phase (11, 12). Additionally, short-term organ cultures of human endometrium, as well as studies using isolated stromal cells, have demonstrated that physiologic concentrations of progesterone or other progestins can suppress MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9 mRNA expression or protein secretion (16, 29, 36, 37). Nevertheless, as is true during estrogen-mediated growth, normal endometrial responses to progesterone require the coordinated actions of numerous paracrine factors during the secretory phase of the menstrual cycle. Under the rapidly changing physiological conditions of early pregnancy, cell-specific MMP regulation in response to rising ovarian progesterone production continues to depend on the positive or negative influences of these locally produced factors (20, 29, 36, 37, 40).

Many MMPs are broadly stimulated by pro-inflammatory cytokines, thus limiting the expression and action of these enzymes during the invasive establishment of pregnancy represents a unique biological challenge. *In vitro* treatments of endometrial stromal cells with onapristone, a PR antagonist, blocks the ability of progesterone to suppress MMP-3 in the presence of IL-1-alpha (29), suggesting that the PR is necessary for suppression of this enzyme when pro-inflammatory cytokines are present. However, we have also demonstrated that pro-inflammatory cytokine production by epithelial cells in co-culture with endometrial stromal cells can act to increase MMP-3 expression by down-regulating PR expression in adjacent stromal cells (41). Taken together, these findings suggest a mechanism by which pro-inflammatory cytokines can act to stimulate focal expression of

MMPs, even in the presence of progesterone. Recently, a down-regulation of PR has been reported during the endometrial window of implantation (42), perhaps related to a need for focal maternal MMP expression at the site of early pregnancy establishment. Interestingly, although a requirement of ligand-bound PR is suggested by the ability of PR antagonist to inhibit progesterone suppression of MMPs in the presence of pro-inflammatory cytokines (29), neither the MMP-3 nor the MMP-7 promoter exhibits classical progesterone response elements (2, 43, 44). However, we have demonstrated that progesterone-mediated suppression of MMP-7 expression by endometrial epithelial cells requires local TGF-beta (40) and *in vitro* treatments of stromal-epithelial co-cultures with a TGF-beta blocking antibody increases secretion of both MMP-3 and MMP-7, even in the presence of progesterone (40, 45). These results indicate that, in addition to adequate levels of PR, the increased expression of TGF-beta during endometrial maturation may be an important component of both cell-type specific progesterone action and cell-cell communication at the maternal-fetal interface.

5. TRANSFORMING GROWTH FACTOR-BETA AND ENDOMETRIAL MATURATION

The focal patterns of MMP expression during estrogen-mediated growth as well as the involvement of cell-cell communication during progesterone-induced MMP suppression indicate that locally produced tissue factors are important regulators of these enzymes across each phase of the menstrual cycle (2, 46). In the human endometrium, progesterone mediates elements of cell-cell communication and tissue maturation by increasing expression of specific growth factors and cytokines, including members of the TGF-beta family. This family of growth factors acts broadly to control both proliferation and differentiation among diverse cell types in healthy tissues as well as in association with disease (47). Among the primary roles of TGF-beta action is the regulation of extracellular matrix (ECM) composition, often acting to inhibit the expression of matrix degrading enzymes while stimulating expression of ECM proteins and MMP inhibitors (48). The cellular effects of TGF-beta are transduced via two serine/threonine transmembrane receptors (TGF-betaRI and TGF-betaRII). Each TGF-beta isotype binds the type II receptor, which then recruits the type I receptor, resulting in activation. Once activated, the receptors initiate a signaling cascade via phosphorylation of smad proteins (49). However, TGF-beta2 has only low affinity for the type II receptor, but has high affinity for the non-signaling type III receptor (betaglycan), which acts to potentiate ligand binding to the type II receptor. In the human endometrium, betaglycan expression has been demonstrated predominately in endometrial stromal cells with the highest levels present during the early secretory phase and during pregnancy (50). The specific expression of both TGF-beta2 and betagylcan at the maternal-fetal interface may be important for immunomodulation since TGF-beta2 is known to inhibit the actions of pro-inflammatory cytokines (51). However, an equally important role for TGF-beta2 production during stromal decidualization may be the suppression of maternal MMPs (40), perhaps related to the suggested ability of this growth factor family to limit trophoblast invasion (52). The specific role(s) of different TGF-beta isotypes in the establishment or maintenance of a maternal-fetal environment which limits MMP expression remains unclear; nevertheless, a 50% reduction in placental TGF-beta2 mRNA is associated with spontaneous or induced pregnancy loss in animal studies (53). The expression of TGF-beta1, TGF-beta2 and TGF-beta3 have each been detected during the menstrual cycle (40, 54); however, studies in humans and primates further indicate that TGF-beta2 expression is most closely aligned with progesterone-mediated endometrial maturation (40, 55, 56). Importantly, a specific lack of endometrial TGF-beta2 expression is associated with infertility in primates (57) and early loss of pregnancy in humans (58).

Although endometrial responses to progesterone appear temporally associated with the expression of TGF-beta2 during the secretory phase of the menstrual cycle, the specific mechanism(s) by which progesterone mediates the expression of this growth factor remains unclear. The regulation of individual TGF-beta genes is complex, and the expression of individual isoforms can be impacted by the presence or absence of numerous other growth factors and cytokines (59). As is true for the MMP promoters, a progesterone response element has not been identified within the TGF-beta2 promoter or 5' flanking region and it is currently unknown whether or not the PR can interact directly with the TGF-beta2 promoter (60). However, glucocorticoid response elements have been found within the human TGF-beta2 promoter (61)and it is possible that the PR acts via binding to these elements, as shown in other systems (62, 63). Alternatively, it is possible that progesterone induction of TGF-beta2 in the endometrium may be principally modulated by the secondary action of other locally produced factors. In this regard, retinoic acid has been shown to specifically induce TGF-beta2 mRNA expression, increase TGF-beta2 mRNA stability, and stimulate the conversion of latent TGF-beta2 to the active form (60, 64-67). Interestingly, TGF-beta2 can act as a downstream effector of retinoic acid signaling and pups born to Vitamin A deficient mice have many of the same developmental defects as TGF-beta2 knockout mice (64, 68). Retinoic acid is the active form of Vitamin A and nutritional deficits of this essential vitamin have been associated with infertility (69, 70). The studies discussed above clearly indicate that, in response to progesterone, an appropriate expression of locally active factors is a requirement for endometrial maturation and normal fertility.

6. COOPERATIVE INTERACTIONS BETWEEN PROGESTERONE, RETINOIC ACID AND TGF-BETA

In all mammals, retinoic acid is critical in the maintenance of epithelial morphology, as well as the differentiation and function of multiple organ systems. During embryonic development, numerous mesenchymal-epithelial interactions are dependent on the local action of both retinoic acid (71, 72) and TGF-beta2 (68) for normal organogenesis and function. Since the cyclic growth and maturation of the adult endometrium in primates and humans represents a development-like process, it is not surprising that retinoic acid and TGF-beta serve interactive roles in regulating stromal-epithelial interactions in response to steroids. In the adult human endometrium, both types of nuclear retinoid binding receptors, RAR and RXR, have been detected, although the lack of cyclic

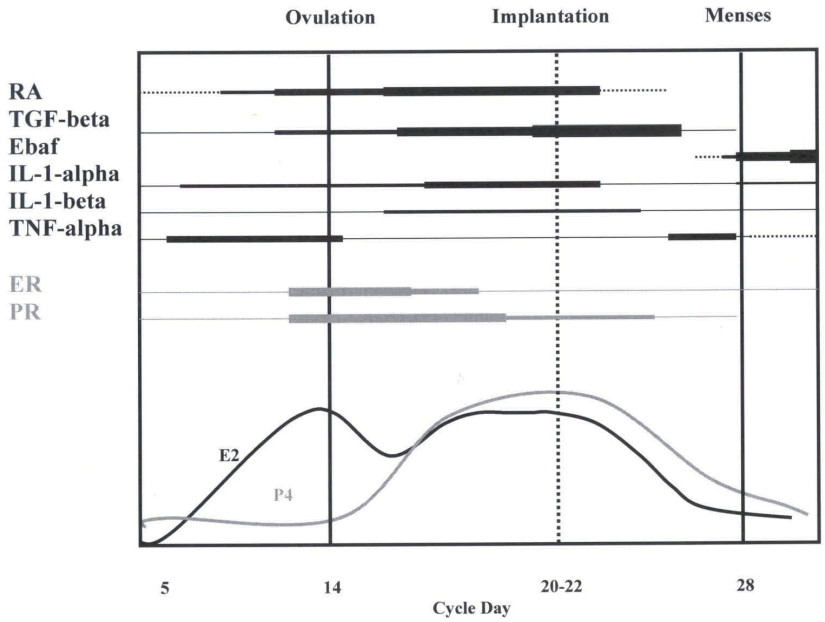

Figure 2. Summary of the expression pattern of selected cytokines, growth factors and steroid receptors during the menstrual cycle. Intensity of expression is indicated by thickness of bar. Relative levels of estradiol (E2) and progesterone (P4) are also indicated.

variation (73, 74) suggests that ligand availability may dictate cycle dependent endometrial responsiveness to retinoic acid. Specific binding proteins mediate tissue retinoid transport and metabolism, including the cellular retinoid binding proteins (CRBP I and CRBP II) and the retinoic acid binding proteins (CRABP I and CRABP II), the latter of which appears to be cyclically regulated in the human endometrium (75, 76). During a previous collaborative effort, rat stromal cells were found to develop the capacity to synthesize retinoic acid during the process of decidualization (77, 78). In light of the potential importance of the retinoid system in humans, we subsequently examined whether retinoic acid and TGF-beta serve interactive roles as local mediators of progesterone action during endometrial maturation. Supporting the rodent findings, we showed that progesterone-mediated retinoic acid synthesis occurs during *in vitro* decidualization of human stromal cells (30). Together these findings suggest that retinoic acid may serve an important role during pregnancy, and that a principal site of action may be at the maternal-fetal interface. To approach this possibility, we examined the ability of retinoic acid to regulate the cell-specific expression of MMP-3 and MMP-7 in endometrial stromal and epithelial cells respectively as well as the role of TGF-beta as a mediator of retinoic acid action. Similar to our previous findings with progesterone, we found that *in* vitro treatments of human cells with retinoic acid suppresses MMP-3 and MMP-7 expression, although this action can be blocked by co-treatments with a pan-specific TGF-beta blocking antibody (10, 45). Our results to date strongly suggest that TGF-beta is a critical secondary signal for the regulation of endometrial MMPs, acting in concert with both progesterone and retinoic acid during endometrial preparation for pregnancy.

In the absence of successful pregnancy, a recently described natural inhibitor of TGF-beta, EBAF (Endometrial bleeding Associated Factor), appears to be involved in promoting rapid increases in MMP expression at menstruation (46, 79). In response to increasing EBAF expression, the actions of TGF-beta are inhibited, perhaps allowing for a more rapid induction of MMP expression than would occur with progesterone withdrawal alone. Interestingly, the ability of EBAF to increase MMP-3 and MMP-7 expression in short-term endometrial organ cultures can be blocked by pretreatment with progesterone (80), similar to the ability of progesterone to prevent induction of MMPs by pro-inflammatory cytokines (29, 30). At present, the molecular mechanism by which progesterone blocks the stimulation of MMPs in the presence of either EBAF or pro-inflammatory cytokines is unknown. However, in non-reproductive tissues, both retinoic acid and TGF-beta have been reported to antagonize pro-inflammatory cytokine stimulation of MMPs (81, 82) while retinoic acid has been shown to regulate the expression of EBAF (83, 84). Efforts are currently underway in our laboratory to further examine the co-operative regulation of endometrial MMPs by progesterone, retinoic acid and members of the TGF-beta family. A key goal of these studies is to determine the role of these factors in creating a unique environment at the maternal-fetal interface which regulates MMP expression and action during establishment of pregnancy.

7. PERSPECTIVE

The complex and interactive system required for normal endometrial MMP regulation in response to either estrogen or progesterone provides for a remarkable biological balance, perhaps important as a "buffer" to prevent the overexpression of these enzymes in the highly steroid-sensitive endometrium. In response to changing levels of ovarian steroids, numerous growth factors and cytokines are expressed in the endometrium across each menstrual cycle (figure 2).

Exploring the regulatory actions of locally produced growth factors and cytokines on members of the MMP family and their inhibitors will allow a better understanding of the normal steroid physiology of the human endometrium. As noted in our above dicussions, exposure of human endometrium to progesterone induces a unique endometrial environment which regulates local, cell-specific responses to pro-inflammatory cytokines produced at the maternal-fetal interface (29, 30). When pregnancy does not occur, falling levels of ovarian progesterone trigger a cascade of vascular and tissue changes, involving the action of factors such as EBAF and pro-inflammatory cytokines which promote broad MMP expression leading to menstruation (2, 85). During the window of implantation, a lack of normal progesterone action or expression of progesterone-induced local tissue factors could easily lead to aberrant MMP regulation, endometrial instability and a loss of fertility.

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