Angionesis and the inception of pregnancy

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STEROIDS AND CYTOKINES IN ENDOMETRIAL ANGIOGENESIS
- review -

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Cyclic regulation of angiogenesis in the endometrium

The cyclic remodeling of the endometrium results in a receptive endometrium, essential for successful implantation and placentation. Angiogenesis (Fig. 1) is needed to support the proliferation and differentiation of endometrial cells after menstruation[9]. The menstrual cycle consists of three consecutive phases: the proliferative phase, the secretory phase and the menstruation (Fig. 2). Angiogenesis occurs during post-menstrual repair and the subsequent thickening of the endometrial tissue. The post-menstrual repair process occurs during the early proliferative phase. The subsequent episode of angiogenesis takes place during the mid-proliferative phase and contributes, in interaction with other tissue cells, to further thickening of the endometrium under the influence of increasing estrogen concentrations. Further adaptation of the newly-formed vessels, including enlargement of the vascular structures, proceeds during the early secretory phase[10]. As a result, spiral (coiled) arteries and a subepithelial capillary complex are developed in the endometrium[11]. During the late secretory and menstrual phase little angiogenic activity is observed[12].

The spiral arteries, which are highly sensitive to ovarian steroids, maintain the blood supply of the functional layer of the endometrium (Fig. 3)[13]. The functional endometrium is shed during menstruation due to the fall in steroid level[14]. The blood supply to the basal layer of the endometrium is *via* the basal (straight) arteries, which are considered to be insensitive to steroidal stimulation[15]. The basal layer gives rise to a new functional layer: new arteries form from arterial stumps left over after menstruation. Endometrial cellular growth and differentiation combined with changes in vascular permeability transform a dense, thin endometrium into a highly edematous, thick endometrium[16].

Markee[13] was the first to demonstrate the steroidal regulation of endometrial angiogenesis. Endometrium was transplanted into the anterior chamber of a rhesus monkey eye and endometrial angiogenic activity was found to parallel the changes in the uterus. Later, Abel[17] observed rapid cyclic vascularisation of human endometrial explants that were transplanted to the hamster cheek pouch. In both models, the extent of vascularisation and incidence of bleeding were influenced by ovarian steroids.

Endothelial cells of the endometrium appear to be highly angiogenic, as can be concluded from studies in our own laboratory and from studies by others which showed that endometrial tissue induced an angiogenic response in the chorioallantoic membrane (CAM) assay. In contrast, endothelial cells from most other benign tissues did not easily show angiogenic activity[18]. Therefore the endometrium is pre-eminently suited for studying the effects of steroids on the process of angiogenesis.

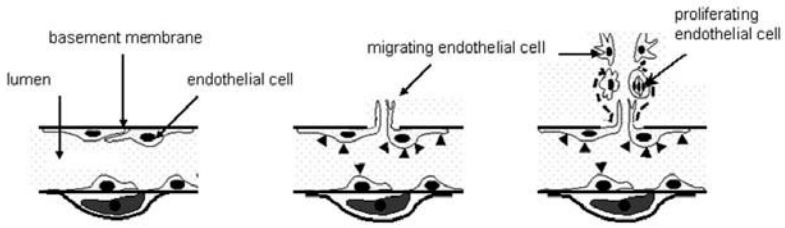

Figure 1. In mature (non-growing) capillaries the vessel wall is composed of an endothelial cell lining and a basement membrane, in which pericytes (blue) usually are present. Angiogenic factors (▲) bind to endothelial cell receptors and initiate angiogenesis. When the endothelial cells are stimulated by angiogenic growth factors, they secrete proteolytic enzymes like metallo proteinases (MMPs) and enzymes of the plasminogen activator (PA) system, which degrade the basement membrane surrounding the vessel. The junctions between endothelial cells are loosened, the cells migrate through the space created, and the newly formed sprouts migrate and proliferate. *[See appendix: color figures]*

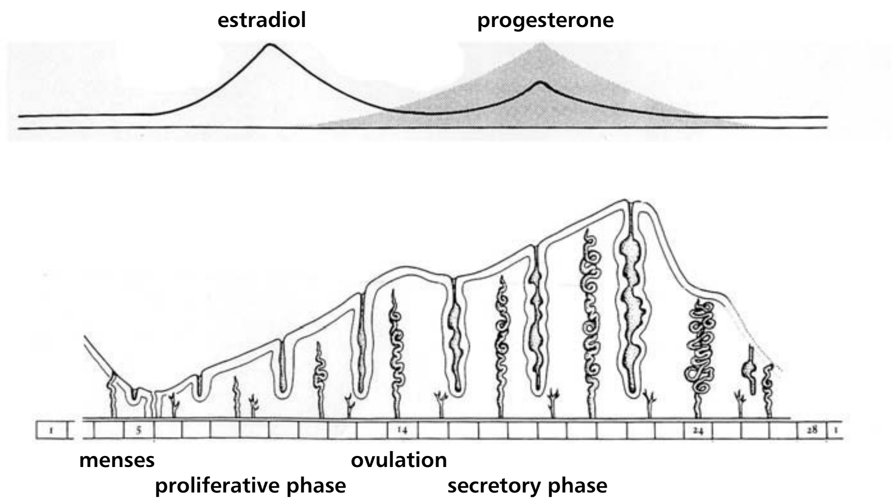

Figure 2. The endometrium undergoes cycles of rapid growth, remodeling, differentiation and angiogenesis, directly or indirectly in response to changes in ovarian steroids. After menstruation increased angiogenesis takes place as a process of post-menstrual repair. During the mid-proliferative phase and early secretory phase further thickening of the endometrium is supported by angiogenesis. As a result elongated, coiled spiral arteries and a subepithelial capillary complex can be seen at the end of the early secretory phase. In the absence of pregnancy, progesterone declines, coincident with breakdown of the functionalis layer, which is expelled with the menstrual flow. Estradiol rises at the end of menstruation, coincident with a new cycle of tissue renewal originating from the intact basal layer.

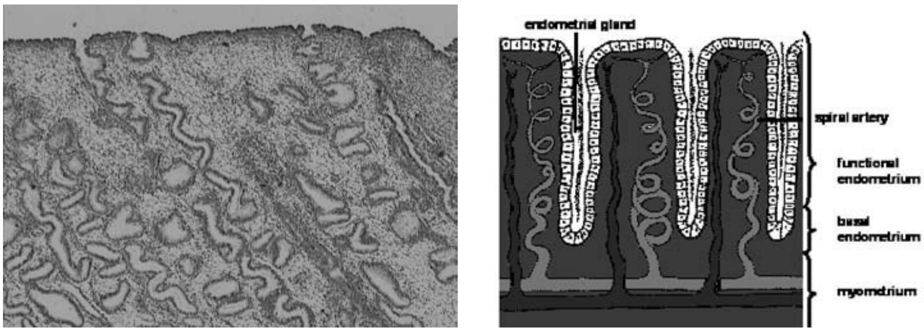

Figure 3. After menstruation from the basal endometrium a new functional endometrium grows. The basal arteries give rise to new blood vessels, which will form spiral arteries and a subepithelial capillary complex. Together with stromal and epithelial growth and differentiation, and increased vascular permeability, an edematous, thick receptive endometrium is prepared for implantation. *[See appendix: color figures]*

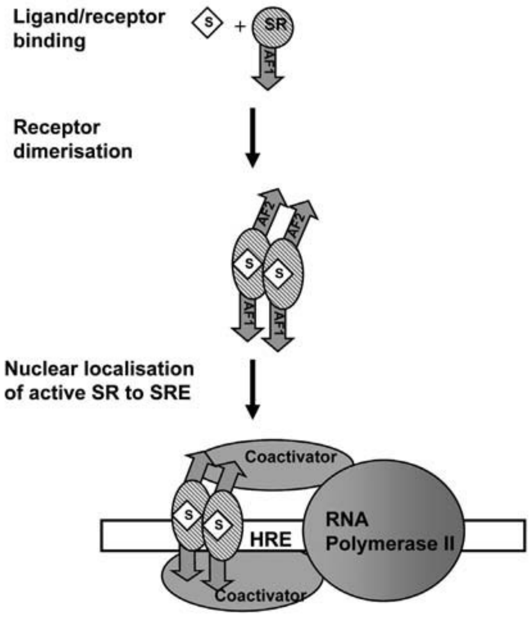

Figure 4. Steroid (S) binding to steroid receptor (SR) initiates a sequence of events including dimerization and binding to DNA sequences, termed hormone response elements (HRE), in the regulatory regions of target genes. The ligand-receptor complex recruits other proteins to the transcriptional complex which act with SR as co-activators or co-repressors of transcription. It is likely that ligand induced conformational changes in the SR influence protein-protein interactions in the transcriptional complex by altering the relative orientation of the independent transcriptional activations domain (AF).

Steroids

Steroid hormones are small lipid-soluble molecules with a structure derived from cholesterol. The steroid hormones include the sex steroids (estrogens, progestagens and androgens; Table 1), glucocorticoids, mineralocorticoids, cholecalciferol, and derivatives. The sex steroids promote sexual function (reproduction) and are responsible for the development of the male and female sexual characteristics. The male sex steroids (androgens, especially testosterone) are produced in the Leydig interstitial cells of the testes and in small amounts also in the ovary and the adrenal cortex. The female sex-hormones estradiol and progesterone are produced in the ovary, the placenta and in small amounts also in the adrenal cortex. Estradiol and progesterone have an important role in the cyclic alterations of the endometrium.

Steroid receptors

Steroids can pass through the cell membrane and bind to specific (nuclear) receptors. The steroid hormone receptors belong to a large superfamily of ligand-regulated nuclear receptors, which share a common structural and functional organization with distinct domains[19]. Nuclear receptors can control the activity of target genes through direct association with specific DNA sequences known as hormone response elements (HREs)[20,21]. The nuclear receptors bind mainly as dimers to the HRE and each monomer interacts with a half-site sequence within the HRE. Receptor-binding specificity is determined by the primary nucleotide sequence, but also by orientation (palindromic or direct repeats) and spacing between the two half sites of the HRE. After binding to DNA, the receptor is thought to interact with co-activators, co-repressors and other transcription factors that link the receptor to components of the basal transcriptional machinery, including RNA polymerase II (Fig. 4).

Two estrogen receptors (ERs) are known, ERα and ERβ. The two ERs are derived from different genes, and appear to have unique tissue distribution and their own sets of specific functions[22,23]. The human ERβ shows homology to ERα especially in the central DNA-binding domain, less in the ligand-binding domain, with only minimum homology in the amino-terminal domain, which has a transactivating function.

It is becoming increasingly clear that the classical model of ER action, *i.e.* ligand-activated ERs interact as homodimers with high-affinity estrogen response elements (EREs) within target gene promoters, is too simple. ERα and ERβ can also interact with DNA sites that do not contain EREs, for example the activator protein-1 site[24]. Also, ERα can modulate gene expression *via* complexing of the transcription factors NF-IL6 and NFκB[25]. Furthermore, estrogens may also act *via* receptors on the cell surface to achieve rapid, non-genomic effects[26,27]. These recent discoveries of several new regulatory mechanisms

Table 1. Sex steroids.

Androgens	Progestagens	Estrogens
5α-Dihydrotestosterone*	Progesterone*	17β-Estradiol*
Testosterone	17α-Hydroxyprogesterone	Estradiol
Androstenedione	20α-Dihydroprogesterone	Estrone
Dehydroepiandrosterone		

*Most effective endogenous steroid at binding and activating its receptor.

Table 2. Relative antiangiogenic potency of steroids.

Compound	Percent potency
Tetrahydrocortisol	100
17α-Hydroxyprogesterone	56
Hydrocortisone	51
11α-Epihydrocortisol	14
Cotexolone	12
Corticosterone	12
Desoxycorticosterone	8
Testosterone	3
Estrone	2
Progesterone	0
Pregnelone	0
Cholesterol	0

Relative antiangiogenic potency of steroids and related compounds in the CAM-assay (J. Folkman and D.E. Ingber 1987)

are probably only a fraction of the many different ways in which ERs can alter cellular functioning.

Progesterone receptor isoforms A and B (PRA, PRB) are the two principal mediators of the biological activities of progesterone in humans and many other vertebrate species. The A- and B- isoforms arise from a single gene and are identical except for the extended N-terminus of B-receptors. Evidence is accumulating that the two isoforms differ extensively in function, suggesting that their ratio of expression may control progesterone responsiveness in target cells[21,28].

Only in recent literature has a distinction been made between ERα and ERβ and PRA and PRB. Consequently, in discussing the older literature we will use the terms ER and PR without specifying which isoform(s) is (are) involved.

In the endometrium epithelial and stromal cells express ERs and PRs[29-33]. The higher uterine expression of ERα as compared to ERβ may suggest that ERα is responsible for mediating the uterotropic response upon estrogen exposure[34]. ERα knockout mice have a uterus that shows a lack of cell proliferation[35] while ERβ knockout mice show diminished reproductive capacity (small litter size, multiple resorbed fetuses)[36]. ERβ seems to act as a modulator of ERα-mediated gene transcription in the uterus (anti-uterothropic); furthermore ERβ is responsible for the down-regulation of PR in the luminal epithelium[36]. PR knockout mice show an inflammatory response to estradiol in the uterus, with no specific differentiation of the endometrial cells (decidual response)[35]. The expression of the ERs and PRs within stromal and epithelial cells varies during the course of the menstrual cycle[30,37]. Estrogen induces ER and PR during the proliferative phase; progesterone has therefore mainly an effect on an estrogen-primed endometrium[35]. In addition, progesterone by itself and steroid withdrawal downregulate the PR and ER expression[31,38]. PR reaches highest concentrations around mid-cycle, and ER around the mid-proliferative phase, correlating with the plasma peak of estradiol and the maximum mitotic rate of the endometrial cells[38,39]. The receptors decrease during the secretory phase[40]. During the late secretory phase, PR disappears from the glandular epithelial cells, but not from the stromal cells[37,38].

Contradictory results have been reported on the expression of ER and PR in endometrial endothelial cells: Iruela-Arispe *et al.*[41] were able to detect ER and PR in endothelial cells, whereas Kohnen *et al.*[32] were not. Therefore, it remains to be established whether the effects of estrogens and progestagens on angiogenesis are directly on endothelial cells or indirectly *via* cytokines derived from estrogen- or progestagen-activated stroma or epithelial cells.

The role of angiogenic growth factors in the endometrium and their regulation by steroids

Polypeptide growth factors are recognized as key regulators of cell proliferation, differentiation and angiogenesis. Several angiogenic factors are synthesized in the endometrium[42,43]. Only those angiogenic factors that have been found (or suggested) to respond to ovarian steroids in the endometrium are discussed. These factors include vascular endothelial growth factor (VEGF), fibroblast growth factors (FGFs), epidermal growth factor (EGF), insulin-like growth factors (IGFs), transforming growth factor α (TGFα), transforming growth factor β (TGFβ), tumor necrosis factor α (TNFα), thymidine phosphorylase, adrenomedullin and erythropoietin (Epo).

Endometrial angiogenesis depends on locally produced growth factors and cytokines, which act in a paracrine way on endothelial cells. It has been generally assumed that these angiogenic growth factors are under steroidal control on the basis of the cyclic variation of these factors, their receptors and regulatory proteins[18,44] and the *in vivo* modulation of these factors by steroids in endometrial tissue[45]. The angiogenic growth factors exert their action by affecting endothelial proliferation and migration/invasion. The latter process depends on the interaction of endothelial cells with their extracellelular matrix, which is controlled by the expression of integrins and matrix-degrading proteases, in particular the plasminogen activator/plasmin system and matrix-degrading metalloproteinases (MMPs). Ample evidence has been provided that the expression of these matrix degrading proteases is under the control of steroids in the endometrium[42,46-51].

Steroids stimulate paracrine VEGF production

A major angiogenic stimulus is the endothelial cell mitogen VEGF. In addition to inducing endothelial proliferation, it modulates the expression of many genes including proteases, it affects endothelial permeability, and it is involved in the maintenance of immature blood vessels[9,52]. Various cell types in the endometrium express VEGF, while estradiol and progesterone appear to regulate its expression. VEGF expression is an important target for estrogens and progestagens in the regulation of angiogenesis in the endometrium.

In the human endometrium, the glandular epithelium and stromal cells produce VEGF, with a higher expression in the glands than in the stroma[53-56]. Stromal macrophages and leucocytes may also be an important source of VEGF[57,58]. VEGF diffuses into the interstitial tissue and binds to capillaries and spiral arteries. The predominant endometrial isoforms of VEGF are the diffusible $VEGF_{121}$ and $VEGF_{165}$, whereas the $VEGF_{189}$ and a $VEGF_{145}$ isoforms are only weakly detectable[16,54,56,59]. Several studies have reported a cyclic or a steroid-dependent variation in the expression of VEGF and VEGF receptors in the endometrium and in isolated endometrial stromal cells[16,29,55,60-65]. This expression pattern, mainly seen in the endometrial stroma, appears to run parallel to the proliferation of blood vessels, *i.e.* the highest in the proliferative and early secretory phase[54,65], although contradictory results has been described[66]. VEGF expression by epithelial cells increased in the secretory and menstrual phase[54,66]. However, it is uncertain whether this VEGF is available for endometrial microvessels, or excreted *via* the glands into the uterine lumen.

VEGF may be an early response gene for estradiol since two sequences have been identified as being homologous to the estrogen response element. These two elements bind both ERα and ERβ specifically, mutations abolish receptor binding, ERα- and ERβ-specific antibodies interact with complexes formed with the corresponding receptor subtypes and transcriptional activity is blocked by the anti-estrogen ICI 182,780. There may be also non-genomic steroid effects, explaining the rapid effects observed[67-69]. Progesterone response elements have not been described[64].

Direct proof of the regulation of VEGF production by estradiol and progesterone was given in a number of *in vivo* and *in vitro* experiments. Both estradiol and progesterone stimulated VEGF expression in the rat and ewe uterus[32,62-64,70-72], at least partly due to an increased VEGF transcription[64,71]. The addition of estradiol and/or a progestagen (methoxyprogesterone acetate (MPA) or progesterone) to isolated stromal or epithelial cells from the human endometrium also increased VEGF mRNA expression significantly[29,59,61,62;66]. In human stromal cells, the exposure to estradiol was accompanied by an increased VEGF secretion into the conditioned medium[59]. An increase in VEGF mRNA was further seen in an endometrial carcinoma cell line after estradiol treatment[54]. The anti-estrogens tamoxifen and nafoxidine have been reported to induce VEGF-A mRNA expression in rodents[71]. These anti-estrogens act in a tissue-specific manner and act as estrogen agonists in the endometrial cells studied. ICI 182,780, thought to be a more general anti-estrogen[16,73], and mifepristone, a progesterone receptor antagonist[66], both blocked the VEGF-A expression.

The high-affinity receptors VEGFR-1 and VEGFR-2 were mainly found on endothelial cells in the endometrium[74]. Endothelial strands, which had not formed a lumen, were strongly stained for both receptors. Rogers *et al.*[55] found the receptors expressed at low levels throughout the cycle with an increase in the menstrual phase. Inhibition of VEGF activity using soluble-VEGFR-1 prevents endometrial maturation[75]. No information is presently available on the steroid regulation of VEGF receptors.

The overall picture that emerges from the VEGF studies is that VEGF expression, especially by the stromal cells, is likely to be under the control of both estrogens and progestagens. It seems that VEGF takes care of the formation of the subepithelial capillary complex during the proliferative phase and the further growth of the spiral arteries in the secretory phase as can be concluded from the immunostaining on these vessels.

Effect of steroids on bFGF synthesis

Fibroblast growth factor-2 (basic FGF, bFGF) regulates the proliferation and differentiation of many cell types[76,77], and is known to stimulate different steps of the angiogenesis process, including endothelial migration, proliferation and invasion, the production of plasminogen activation factors (u-PA and u-PAR) and the induction of $\alpha_2\beta_1$, an integrin with adherent potencies for extra-cellular matrix components[76,78-81]. In the human endometrium bFGF was found to be present at levels higher than those found in other tissues[82,83]. Staining for bFGF was seen in epithelial cells and stromal cells, in basement membranes and smooth muscle cells of medium-sized blood vessels and in endothelial cells and the basal lamina of capillaries[77,84,85]. Its homoloque FGF-1 (acidic FGF) was also found in the human endometrium[84]. bFGF and its receptor increased specifically in the stromal compartment in the proliferative phase and decreased in the secretory phase[85,86]. FGF receptor-1 was detected on endothelial cells throughout the cycle[85].

Estradiol has been found to stimulate bFGF production and excretion by isolated human endometrial stromal cells (fibroblasts) and by endometrial adenocarcinoma cells, whereas progesterone inhibited the estradiol-induced increase in bFGF synthesis but did not affect basal bFGF synthesis[87,88]. Furthermore, estradiol up-regulated bFGF expression in the human, rat and ewe uterus[89] as reviewed elsewhere[55,72]. In addition, the timing of the increased expression of bFGF, as well as that of VEGF, preceded the microvascular growth in ewe uteri[90].

These data suggest that bFGF may be involved in the physiological and tumor angiogenesis of the endometrium and that, similarly to VEGF, with which bFGF acts synergistically[4], the endometrial stroma seems most sensitive to steroidal regulation of bFGF expression. However, the importance of bFGF in endometrial angiogenesis has been disputed by others[83,84].

Tumor necrosis factor α (TNFα)

TNFα is a pleiotropic cytokine that exerts a variety of effects in the endometrium, one of which might be angiogenesis. *In vivo*, TNFα induced capillary blood vessel formation in the rat cornea and the developing chick chorioallantoic membrane at very low doses[91]. *In vitro*, TNFα induced capillary-like structures by bovine adrenal capillary endothelial cells grown on type-1 collagen gels[91]. In human endothelial cells TNFα acted in concert with angiogenic growth factors, such as VEGF and bFGF, and stimulated their angiogenic effect. TNFα had an important stimulatory effect on angiogenesis in this way[4]. However, TNFα alone did not induce the formation of capillary-like structures.

The expression of TNFα and its receptors in the endometrium was markedly affected by the menstrual cycle[92-96]. TNFα was expressed in the endometrium mainly in epithelial cells and further in stromal cells, immunocompetent cells and vascular cells[94,95]. The menstrual cycle-dependency of TNFα in the endometrium, although not always confirmed by all the different studies, suggests that this cytokine is subject to regulation by steroids. Consistently with this suggestion, Tabibzadeh[93] found three half-palindromic estrogen response elements in the promoter region of the TNFα gene. Studies on ovariectomized and hormone-substituted mice showed that uterine TNFα mRNA expression is stimulated by estradiol and progesterone[96]. Progesterone and estradiol caused an increase in TNFα production in cells prepared from the proliferative endometrium[97]. In addition, its peak expression in the proliferative phase and in the early- to mid-secretory phase suggests that TNFα may contribute to endometrial angiogenesis.

Other angiogenic growth factors in the endometrium

Yasuda *et al*.[98] showed estradiol-dependent Erythropoietin (Epo) production in the mouse uterus both *in vitro* and *in vivo*, suggesting cycle-dependent fluctuation of Epo concentrations in the uterine tissue. The induction of the expression of Epo was rapid (similar to that seen with VEGF) after administration of estradiol. The uterine endothelial cells were positive for the Epo-receptor[98,99], which had a low ligand affinity compared with the receptors on erythroid precursor cells. Epo stimulated migration, proliferation and angiogenesis of endothelial cells *in vitro*[99,100]. The fact that Epo plays a role in endometrial angiogenesis was underpinned by the finding that intra-uterine injection of Epo stimulated angiogenesis in the mouse endometrium[98].

Other growth factors that induce angiogenesis in classical angiogenesis models are encountered in the endometrium and are under cyclic control. As such it is likely that they are directly or indirectly affected by sex hormones, but this relationship remains to be elucidated. Thymidine phosphorylase (PD-ECGF) and adrenomedullin have recently been reviewed by Oehler[30]. In addition, epidermal growth factor (EGF)[35,101,102], transforming growth factors-α and -β (TGFα, TGFβ)[61,103-105], and insulin-like growth factors (IGF-I, IGF-II)[106,107] are found in the endometrium, where they probably act as paracrine factors.

Cell-matrix interactions and pericellular proteolysis

An important function of the angiogenic growth factors is to induce endothelial proliferation and thus to increase the mass of available endothelial cells. In addition, the process of angiogenesis involves a number of subsequent and mutually interacting steps. First, the endothelial cells have to penetrate their own basal membrane, to migrate, and to invade in the underlying interstitial tissue. This migration is accompanied by endothelial proliferation. Subsequently, the endothelial cells reorganize into tubular structures and acquire a new basal membrane. Finally the vascular structures become connected with the flowing blood and the new vascular structures become stabilized by interaction with pericytes and paracrine factors (see Fig. 1). Cell-matrix interaction is an important factor in the maintenance of healthy blood vessels and is subject to alterations during migration and invasion of cells. Cell receptors, in particular integrins, and cell-bound matrix remodeling proteases are involved in this process. In addition to their effects on cell migration and invasion, these matrix-degrading proteases are also involved in the degradation of the basal membrane and in lumen formation. These proteolytic activities are mainly exerted by the plasminogen activator (PA)/ plasmin system and the family of matrix-degrading metalloproteinases (MMPs). They are regulated by their expression and the expression of their inhibitors and cellular receptors. It is of interest to note that the expression of many of these proteins is under the control of estrogens and progestagens. It is likely that these proteases show partial redundancy with respect to their function in cell migration and tissue remodeling. This explains why deletion of a single protease, such as urokinase-type plasminogen activator (u-PA)[108], plasminogen[109] or PA inhibitor type-1 (PAI-1)[110] does not gravely interfere with fertility in mice.

Plasminogen activator/plasmin system

The plasminogen activator/plasmin system is a protease cascade, in which plasminogen activators (PAs), *viz.* tissue-type PA (t-PA) and urokinase-type PA (u-PA), activate the plasma protein plasminogen. The subsequently generated plasmin is a broadly acting protease involved primarily in fibrin degradation (fibrinolysis) but also able to degrade matrix proteins directly and to activate several pro-MMPs. Studies *in vitro* and in specific *in vivo* models have shown the importance of the PA/plasmin system in angiogenesis[111,112]. In particular, in a fibrin matrix the formation of capillary-like tubular structures is completely dependent on the cell-bound u-PA and plasmin activities[5]. The ingrowth of such tubular structures was strongly inhibited by testosterone, partly by estradiol and 2-methoxyestradiol but not by progesterone[7]. The effect of testosterone appeared to be related to the expression of u-PA by microvascular endothelial cells[7]. Because these experiments were done with male (foreskin) microvascular endothelial cells, it is possible that the effects of estradiol and progesterone have been underestimated.

The endometrium releases t-PA and u-PA as well as their inhibitor PAI-1[46,48,113]. U-PA and u-PA receptor (protein and mRNA) are found in endometrial stromal cells[114] and endothelial cells[114,141]. Casslen *et al*. however found that epithelial cells also produce u-PA[46]. The maximum release of u-PA from endometrial tissue was during the late proliferative phase[115]. While estradiol did not affect u-PA expression in endometrial tissue cultures and in isolated stromal cells, addition of progesterone, after priming with estrogen, resulted in a reduced secretion of u-PA[46,47]. The release of u-PA by cultured endometrial epithelial cells was not influenced by these steroids[46]. In the secretory phase a lower u-PA activity was seen[47], which could be related to a reduced u-PA activity in endometrial tissue and stromal cell cultures after stimulation with progesterone. The reduced u-PA activity may be caused by an increased expression of PAI-1 in both tissue and stromal cell culture, after the addition of progesterone[48,49]. The increased number of u-PA receptors in the secretory phase[116], which was demonstrated in the cultured stromal cells after adding progesterone, may facilitate the removal of u-PA:PAI-1 complexes and thus keep new receptors available for u-PA interaction[5].

Despite an interesting co-expression of PAs and PAI-1 with early developmental processes, mice that lack u-PA[108], plasminogen[109] or PAI-1[110] have a normal development and are fertile. Therefore, the cell-bound u-PA and plasmin-dependency of angiogenesis is probably conditional, *i.e.* it occurs only under specific conditions, such as in fibrin-rich wound matrices. Even in such conditions a rescue system exists. Hiraoka *et al*.[117] recently demonstrated that in plasminogen-deficient conditions, MMP activity could serve as a fibinolysin and substitute for plasmin activity in fibrinolysis and angiogenesis in the mouse.

MMPs

MMps are a highly regulated family of enzymes, which together can degrade most components of the extracellular matrix. They play a role in the menstrual bleeding, tissue degradation, and reorganization and repair within the endometrium.

MMPs are expressed in the endometrium in cell-type and cycle-specific patterns, consistent with regulation by steroid hormones[118]. One may suggest that the MMPs, which are present in the late secretory phase and during menstruation, play a role in the tissue degradation leading to the breakdown of the endometrium[119,120]. The MMPs, which are abundant in the proliferative and early secretory phase, might play a role in the endometrial growth, remodeling and angiogenesis. However, no direct proof has been given yet regarding their involvement in endometrial angiogenesis and little is known about their regulation by steroids in the proliferative and early secretory phase.

MMP-1, MMP-2, MMP-7 and MMP-11 were expressed in the proliferative phase, while MMP-3 and MMP-9 were occasionally present[118,121]. MMP-2 showed staining in the stromal cells and vessels[121], MMP-9 expression was seen in leucocytes and spiral arteries and in the early secretory phase in the epithelium[121], while MMP-1 and MMP-3 were present in vascular structures[121]. MMP-7 and MMP-11 appeared restricted to the epithelium and stromal cells, respectively[118]. In isolated stromal cells progesterone inhibited the expression of pro-MMP-7, pro-MMP-11 and pro-MMP-3[51]. There is relatively little cyclical variability of the specific tissue inhibitors of metalloproteinases (TIMPs) TIMP-1 and TIMP-2: both were strongly expressed in endometrial vessels throughout the cycle[121].

Inhibitors of angiogenesis

Because angiogenesis plays an important role in physiological and pathological processes, stimulation and inhibition of angiogenesis could hold a potential therapeutic application. While angiogenesis is stimulated by the application of angiogenic growth factors, inhibition of angiogenesis can be achieved both by naturally occurring angiogenesis inhibitors and by exogenously applied compounds. Among naturally occurring anti-angiogenic factors are thrombospondin-1[33], platelet factor-4, interferons, vascular endothelial growth inhibitor and 2-methoxyestradiol[122]. In addition, anti-angiogenic products can be formed by proteolysis of natural proteins, such as angiostatin (peptide derived from plasminogen)[123] and endostatin (fragment of type XVIII collagen)[124]. Pharmacological inhibitors comprise amongst others thalidomide[125], AGM-1470, a fumagillin derivative[126], VEGF receptor antagonists, inhibitors of MMPs[127] and the amino terminal fragment of u-PA[128].

Thrombospondin-1 (TSP-1)

TSP-1 has been reported to inhibit angiogenesis *in vivo* and *in vitro*[129,130]. TSP-1 was predominantly found in the basement membranes of glands, in small blood vessels and capillaries and diffusely in the stroma of the functional, secretory endometrium[33]. Its expression seems to coincide with the suppression of angiogenesis.

TSP-1 expression and secretion appeared to be stimulated in isolated endometrial stromal cells by progesterone and not by estradiol, with the effect being blocked by anti-progestin[33]. In the human TSP-1 gene two progesterone-responsive elements were found in the promoter[33,131]. It remains to be seen whether these sites are functional and responsible for progesterone-induced effects.

Angiostatic steroids

Angiostatic behavior has been reported for some steroids, whether or not in the presence of heparin or heparin derivatives[7,8,132-135]. Angiogenesis inhibition by steroids was said to be caused by the dissolution of the basement membrane in regressing capillaries, by decreasing endothelial cell proteolytic capacity and/or by disrupting microtubules in endothelial cells[7,8,122,136]. When combined with heparin the inhibition was independent of the anticoagulant activity of heparin. Also, the glucocorticoid and mineralocorticoid activity of the steroids did not play a role[132].

Table 2 shows several steroids that inhibit angiogenesis in combination with heparin, together with their anti-angiogenic potency[8]. Testosterone, dexamethasone, methoxyestradiol, cortisol, 17α-hydroxyprogesterone, medroxyprogesterone acetate, androstenedione and tetrahydro S also exert inhibitory effects on angiogenesis *in vitro* in the absence of heparin [7,122,135,137,138]. The effect of methoxyestradiol was also demonstrated *in vivo*[122,135,138]. Jaggers *et al.*[137], using very high concentrations of estradiol, found a complete inhibition of angiogenesis in a rat aorta explant assay. Reports on effects of estradiol and progesterone on angiogenesis are conflicting, but most studies agree that neither estradiol nor progesterone appears to have intrinsic angiogenic activity[8,132,139].

Conclusion & perspectives

From the preceding data pictures emerges in which estrogens and progestagens act on endometrial cells, in particular stromal and epithelial cells, and induce paracrine factors that stimulate angiogenesis in the endometrial vessels. Whether these steroids also act directly on endometrial endothelial cells remains uncertain. The data provide insight into the molecular mechanisms underlying the original finding of Markee of steroidal regulation of endometrial angiogenesis. The paracrine interaction between endometrial cells may also explain why it has been difficult to demonstrate in cultured cells *in vitro* the obvious effects of steroids on the endometrium seen *in vivo*. The interaction between different tissue cell types appears to be essential for observing the effect of the steroids. In addition to the paracine regulation or the production of angiogenic growth factors by steroids, the endothelial response is also influenced by other, non-steroid regulators, such as growth factors/cytokines, prostaglandins, and hypoxia. The elucidation of the various interactions between endometrial cells upon challenge by steroids and other factors will clarify how steroids act on endometrial angiogenesis.

Insight into the regulation of angiogenesis in the endometrium by steroids might contribute to the understanding of the effects of steroids and their derivatives on angiogenesis in tumors. Many of the angiogenic factors discussed have also been shown to play an important role in tumor angiogenesis. In tumors that are affected by steroids, such as breast cancer, angiogenesis might very well be regulated by steroidally-induced angiogenic factors. These factors may act in addition to the direct effect of the steroids on the tumor cells themselves. Knowledge of the factors important in regulating tumor angiogenesis by steroids, and understanding in greater detail the cell-specific expression of steroid receptors and coactivator proteins involved in the induction of gene expression by these steroids may lead to a more effective treatment of cancer with fewer side-effects. In this respect one not only has to take into account tumor-specific expression of receptors and related proteins, but also organ-specific characteristics of endothelial cells[140]. Understanding the mechanisms involved in the local action of specific steroids may provide a rational approach for inducing or inhibiting angiogenesis by steroids or (anti-)steroid derivatives.

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