Histol Histopathol (2004) 19: 167-172

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Angiogenic factors and the endometrium following long term progestin only contraception

C.J. Lockwood, F. Schatz and G. Krikun

Department of Ob/Gyn, New Haven, CT, USA

Summary. Endometria from long term progestin only contraceptive-treated patients display abnormally enlarged blood vessels that are prone to bleeding as well as inflammation and possibly local diminution in blood flow. Such bleeding is also characterized by focal reductions in the expression of angiopoietin-1, a vessel stabilization and maturation agent, and excess production of the potent angiogenic agents, vascular endothelial growth factor and angiopoietin-2. In addition, tissue factor, the key initiator of hemostasis may play an angiogenic role either directly or via the activation of thrombin. This review article summarizes current findings related to the endometrial dysregulation of angiogenic/hemostatic agents following treatment with long term progestin only contraception. Studies in this area offer a promising avenue to alleviate abnormal uterine bleeding associated with this otherwise highly effective form of contraception

Key words: Contraception, Uterine Bleeding, Angiogenesis, Endometrium

Long term progestin only contraception results in abnormal uterine bleeding

Abnormal uterine bleeding (AUB) is the leading indication for discontinuation of long-term, progestin-only contraceptives (LTPOCs) (Belsey, 1988; Su-Juan et al., 1994). These agents provide a highly practical solution to family planning needs in developed and developing countries (Archer et al., 1996; Fan and Su-Juan, 1996). While the biochemical processes underlying LTPOCs associated bleeding are not clearly understood, it is clear that the mechanisms involved diverge from the tight regulation of menstrual cycle-related endometrial hemostasis, angiogenesis and bleeding.

Offprint requests to: Dr. Graciela Krikun, Yale University School of Medicine, Dept. Ob/Gyn, 333 Cedars St., PO Box 208063, New Haven, CT, 06520-8063, USA. Fax: (203) 785-4713. e-mail: graciela.krikun@yale.edu

During the regular menstrual cycle, progesterone (P4) induces estradiol (E2)-primed human endometrial stromal cells (HESCs) to decidualize around blood vessels in the mid-luteal phase in preparation for the implanting blastocyst. Decidualized HESCs are ideally positioned to mitigate the risk of hemorrhage at this time, or to mediate menstruation in response to P4 withdrawal in non-fertile cycles (Lockwood and Schatz, 1996).

Following hormone withdrawal and menstruation, the functional endometrial layer is replenished by the sequential effects of E2 and P4. During menstruation, loss of the superficial endometrial layer produces gaping vessels from which endothelial cells sprout, and recruit pericytes to form capillaries and smooth muscle cells to form larger vessels (Smith, 1998). Peri-menstrual hypoxia is thought to initiate angiogenesis by inducing vascular endothelial cell growth factor (VEGF), the primary angiogenic agent, in endometrial stromal and epithelial cells (Popovici et al., 1999; Sharkey et al., 2000).

By contrast, LTPOC-associated AUB is spontaneous, focal and transient, with stable or elevated circulating P4 levels (Runic et al., 1997). Such AUB appears to be derived from structural changes in the vasculature resulting in dilated, superficial., thin walled "fragile" microvessels (Hickey et al., 1996, 1999; Rogers, 1996; Hickey et al., 1999; Runic et al., 2000). In addition to changes in the structural integrity of the microvessels, changes may also occur in support cells like the pericytes as demonstrated by the reduction in vascular smooth muscle α-actin expression in LTPOC-exposed endometrium (Hickey and Fraser, 2000; Hague et al., 2002). These observations strongly suggest that LTPOC treatment results in aberrant endometrial angiogenesis.

Recent reports (Rogers, 1996; Runic et al., 1997; Lebovic et al., 2000; Hague et al., 2002; Krikun et al., 2002) link LTPOC-associated AUB to aberrant expression of endometrial hemostatic and angiogenic factors. Below we review the current data on this subject.

Vascular endothelial growth factor expression following LTPOC

The human vascular endothelial growth factor (VEGF) gene contains 8 exons, 7 introns and a 14 kb coding region. Alternative exon splicing of a single VEGF gene produces 6 different isoforms. These include the predominant isoform VEGF165, as well as VEGF121, VEGF145, VEGF183, VEGF189 and VEGF 206 (Ferrara, 1999; Robinson and Stringer, 2001). The prominent VEGF isoforms expressed by the endometrium and by primary cultured endometrial stromal cells are VEGF165 and VEGF121 a nonheparin-binding acidic protein, which is freely diffusible (Torry et al., 1996; Ferrara, 1999; Robinson and Stringer, 2001). Binding of VEGF to the Flt-1 and KDR surface receptors activates their tyrosine kinase function resulting in enhanced endothelial cell proliferation, migration, vascular permeability, and protease activity (Ferrara, 1999). Flt-1 and KDR are restricted to endothelial cells, and cells of common embryonic origin (Ferrara, 1999). Binding to both receptors is required for maximal angiogenesis. Expression of Flt-1 in both quiescent and proliferating endothelial cells (Peters et al., 1993) suggests that VEGF regulates endothelial cell maintenance as well as angiogenesis.

Under normal physiological conditions, there appears to be a complex interplay between hormones and VEGF expression in the primate endometrium.

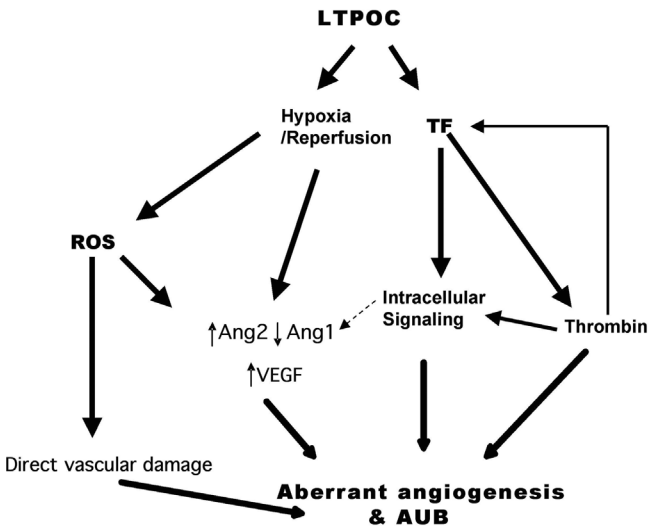

Fig. 1. Pathways of aberrant angiogenesis following LTPOC. LTPOC-induced dysregulation of endometrial blood flow results in hypoxia/reperfusion generating reactive oxygen species (ROS) that can directly damage endometrial vessels or result in decreased expression Ang-1 and enhanced expression of Ang-2 and VEGF. Furthermore, LTPOC results in the focal induction of TF to directly activate intracellular signaling cascades or resulting in the consequential formation of thrombin. Together, these mechanism lead to the formation of fragile vessels that are prone to bleed.

While the VEGF promoter contains 3 half palindromic sites for the estrogen receptor, no progesterone receptor sites have been described (Tischer et al., 1991). There is conflicting data from different laboratories regarding the induction of VEGF expression in the endometrium during the menstrual cycle. Hence, while some reports indicate that VEGF-expression is enhanced in the secretory phase, others have found biphasic increases in the mid and late proliferative and secretory phases of the cycle (Shifren et al., 1996; Smith et al., 1996; Smith 1998, Charnock-Jones et al., 2000).

A study by Zhang et al. (1998) reported on the expression and localization of VEGF during the menstrual cycle. The authors reported that VEGF expression occurred predominantly in the endometrial epithelium and while weak in the proliferative phase, was strong in the secretory phase. VEGF expression in the stroma was weaker than in the glands and did not change throughout the cycle. Conversely, Torry and Torry reported that endometrial stromal cells display very intense foci of VEGF staining in particular during the proliferative phase of the cycle (Torry and Torry, 1997). Finally, a study by Hornung and coworkers reported that while endometrial VEGF expression displays diffusely positive messenger RNA and protein expression, glandular epithelium showed focal VEGF immunostaining at the apical surface (Hornung et al., 1998). The authors propose that VEGF is secreted apically into the gland lumina, and rather than playing an angiogenic role in the endometrium, it is potentially involved in influencing the nutrition and/or apposition of the developing blastocyst (Hornung et al., 1998).

Although physiological VEGF levels promote angiogenesis, over-expressed VEGF induces endothelial vascular "leakiness", bleeding and perivascular ECM dissolution associated with pathological conditions. Indeed, Yokoyama and coworkers have shown that over-expression of VEGF, and 2 of its receptors were related to poor prognosis of patients with endometrial carcinomas (Yokoyama et al., 2000).

Alterations in the production or distribution of VEGF have been proposed to be related to LTPOC-induced AUB. One study demonstrated that the glandular and stromal VEGF staining indices were significantly higher in endometria following Norplant treatment than in untreated endometria, however, no correlation was found between the VEGF staining index following Norplant treatment and endometrial microvascular density (Lau et al., 1999). By contrast, in women treated with Implanon, only the stromal VEGF staining indices were found to be significantly elevated compared to controls and a positive correlation was observed between stromal-VEGF immunoreactivity and endothelial cell density (Charnock-Jones et al., 2000).

An *in vitro* study by Classen-Linke et al. demonstrated that the progestin medroxyprogesterone acetate (MPA) increased VEGF release in endometrial epithelial cell cultures (Classen-Linke et al., 2000). Interestingly, a recent study from our laboratory showed

that MPA did not alter secreted VEGF levels in cultured human endometrial epithelial cells or human endometrial stromal cells. Instead, the study demonstrated that the combined effects of excess thrombin and MPA resulted in the induced expression of VEGF in human endometrial stromal cells but the production of VEGF by glandular epithelial cells was not affected under these conditions (Lockwood et al., 2002). Hence, it is possible that *in vivo*, continued progestin exposure results in the production of other intermediate agents such as thrombin or the expression of other angiogenic agents (see angiopoietins, below) which either result in enhanced endometrial VEGF production or act in concert with VEGF leading to the aberrant angiogenic patterns observed in LTPOC users.

Angiopoietin expression following LTPOC

While VEGF has been identified as the primary vasculogenic and angiogenic factor, recently another family of proteins, the angiopoietins, has also been shown to be a key regulator of angiogenesis. Angiopoietin 1 (Ang-1) and angiopoietin 2 (Ang-2) are glycosylated, secreted proteins which have characteristic protein structures that contain coiled coil domains in the N-terminal portion and fibrinogen like domains in the C-terminal portion of the molecule (Suri et al., 1996; Maisonpierre et al., 1997). Ang-1 was mapped to the long arm of chromosome 8 while Ang-2 was mapped to the short arm of chromosome 8 (Cheung et al., 1998). The four members of this family (Ang-1 to 4) modulate angiogenesis by activating or blocking activation of their endothelial receptor Tie2, a surface receptor tyrosine kinase (Gale and Yancopoulos, 1999; Holash et al., 1999; Valenzuela et al., 1999). Ang-1 affects vascular integrity by acting at a later stage of vascular development than VEGF. Ang-1 always asserts its effects by activating Tie2 (Davis et al., 1996).

Transgenic Ang-1 and Tie2 knockout mice result in embryonic lethality associated with aberrant interactions between endothelial cells and their surrounding supporting cells (Suri et al., 1996). Moreover, transgenic over-expression of Ang-1 elicits marked pathological hypervascularization and regional varicosities (Suri et al., 1998) similar to that observed in LTPOC-exposed endometria.

Unlike the activating role of Ang-1, Ang-2 generally blocks Tie2 activation (Ward and Dumont, 2002). Recent studies have now suggested that Ang-2 has a dual function in the processes of postnatal angiogenesis and vascular remodeling and that Ang-2 signals are required for the proper development and function of the lymphatic vessels (Gale et al., 2002).

Consistent with the action of Ang-2 as a partial antagonist of Ang-1/Tie2 interactions, transgenic over-expression of Ang-2 results in embryonic lethality with a phenotype similar to mice lacking either Ang-1 or Tie2 (Maisonpierre, 1997). Ang-2 is expressed in areas undergoing vascular remodeling (Maisonpierre, 1997). Like VEGF, Ang-2 enhances vascular permeability. Both hypoxia and VEGF up-regulate Ang-2 expression in bovine microvascular endothelial cells (Oh et al., 1999). Thus, VEGF, Ang-1 and Ang-2 have complementary roles in vascular development and maintenance. While many studies have focused on the role of Ang-1 and Ang-2, murine Ang-3 and human Ang-4 have only recently been cloned and their function and localization is not yet well defined (Valenzuela et al., 1999).

Our group has shown that Ang-1 and Ang-2 are expressed in normal human endometrium. Specifically, immunohistochemical studies in the endometrium revealed that both secretory and proliferative endometrium displayed low intensity staining for Ang-1. The highest degree of staining occurred in the endothelium with low level staining in the stroma and in the glands (Krikun et al., 2002). Semi-quantitative RT-PCR studies with isolated stroma or endothelial cells however showed that Ang1 mRNA was only found in the stroma and not in the endothelial cells. We posit that the immuno-histochemical staining for Ang-1 in the endothelial cells results from binding to the receptor Tie-2 and not from synthesis by these cells.

By contrast, the immunohistochemical expression of Ang-2 and Tie2 was absent from the glands, low in stromal cells, and intense in the endothelial cells. The phase of the menstrual cycle did not appear to affect the expression of Ang-2 or Tie2 (Krikun et al., 2000) although high levels of immunohistochemical reactivity precluded a quantitative assessment of the expression of these proteins.

Most interestingly, aberrant expression of Ang-1 and 2 were shown to be associated with pathologic conditions. Recent findings suggest that LTPOC-induced endometrial bleeding occurs as a result of hypoxia/re-perfusion induced free radicals that directly damage vessels and alter the balance of Ang-1 and 2 to produce the characteristic enlarged, and permeable vessels that are prone to bleeding. *In vitro* studies utilizing isolated human endometrial endothelial cells (HEECs), the most relevant model for endometrial microvascular biology studies, demonstrated that both hypoxia and the inflammatory agent phorbol-myristate-acetate, enhanced Ang-2 mRNA levels in HEECs suggesting that Ang-2 plays a role in endometrial pathologies complicated by impaired blood flow and inflammation. (Krikun et al., 2000).

LTPOC exposed endometrium displayed focal, intermittent staining for Ang-1 in the stroma with some areas of high staining and some areas of low to no staining. Hickey et al. (2000) have shown a trend towards reduced endometrial perfusion in LTPOC users suggestive of localized tissue hypoxia. Tissue hypoxia is almost invariably followed by re-perfusion-induced free radical damage. We hypothesized that the patchy Ang-1 endometrial staining following LTPOC was a result of focal hypoxia-reperfusion in these tissues. Consistent with this hypothesis we showed that both hypoxia and oxygen radical production decreased expression of Ang-

1 mRNA and protein in cultured HESCs (Krikun et al., 2002). In addition, it was shown that tissues derived from LTPOC treated women displayed enhanced phosphorylation of the stress activated MAP kinases, suggestive of hypoxia and oxidative damage (Krikun et al., 2002). Focal areas of hypoxia may also help to explain the discrepancies observed in VEGF expression following LTPOC use. Hence, only focal areas exposed to hypoxia would result in focal VEGF induction.

These findings suggest that aberrant angiogenesis and AUB in LTPOC-users may occur due to the dysregulated expression of the key angiogenic factors Ang-1 and Ang-2 in concert with VEGF expression.

Tissue factor expression following LTPOC

In addition to the effects from the classic angiogenic agents described above, studies from our laboratory have suggested that tissue factor (TF), the key initiator of the hemostatic cascade, may be involved in aberrant angiogenesis following LTPOC. TF is a 46 kDa cell membrane-bound glycoprotein resembling the members of the class 2 cytokine receptor family. It is comprised of a hydrophilic extracellular domain, a hydrophobic transmembrane domain, and a cytoplasmic domain (Guha et al., 1986; Bach, 1988; Nemerson, 1988). TF is located on the surface of several cell types including monocytes, smooth muscle cells, fibroblasts and keratinocytes and endometrial stromal cells (Lockwood et al., 1993a; Pendurthi and Rao, 2002; O'Reilly et al., 2003). By contrast, under physiological conditions, TF is absent in cells which are in contact with blood such as the endothelial cells. Its expression however, can be induced in these cells by inflammation, thrombin and angiogenic agents such as VEGF (Pendurthi and Rao, 2002; O'Reilly et al., 2003; Stenina, 2003). Following vascular disruption, perivascular cell-bound TF binds to plasma-derived factor VII or its activated form, FVIIa, to directly activate factor X. Factor Xa complexes with its cofactor Va, to generate thrombin (Bach, 1988; Nemerson, 1988). Cytokines, growth factors, and serum transiently (1- 4 hours) induce TF mRNA, and protein in cultured cells from diverse tissues (Donovan-Peluso et al., 1994; Moll et al., 1995; Cui et al., 1996). By contrast, studies in our laboratory found that *in-vivo* and *in-vitro* decidualization of human endometrial stromal cells was associated with chronically enhanced TF protein and mRNA expression (Lockwood et al., 1993b, 2001). Moreover, enhanced TF expression required progesterone receptors and mediation by the epidermal growth factor receptor (Lockwood et al., 2000).

In addition to hemostasis, TF is now known to mediate tumor invasion, sepsis, inflammation, atherosclerosis and angiogenesis (Ruf and Mueller, 1996; Shoji et al., 1998). The interaction of TF-FVIIa elicits a variety of intracellular signaling events that may be implicated in these processes and include the sequential activation of Src-like kinases, MAP kinases, protein kinase C, PI3-kinase and calcium signaling (Versteeg et al., 2001; Peppelenbosch and Versteeg, 2001). Additionally, the angiogenic role of TF may be mediated by generating thrombin, which binds to and cleaves the endothelial cell thrombin receptor (PAR-1) (Tsopanoglou and Maragoudakis, 1999; Browder et al., 2000) to directly promote human endometrial endothelial cell mitosis (Senger et al., 1996). Moreover, thrombin itself has been shown to be angiogenic and capable of activating pro-angiogenic proteases (Senger et al., 1996). Interestingly, thrombin has also been shown to induce the expression of the VEGF receptors KDR and Flt-1 in endothelial cells (Maragoudakis et al., 2002).

In apparent contradiction with the classical role of TF as the primary initiator of hemostasis, 12 months of Norplant treatment resulted in enhanced TF expression at bleeding sites which also displayed enlarged, distended vessels (Runic et al., 2000). These effects could reflect various signaling mechanisms by TF including indirect signaling by thrombin. Indeed, as mentioned above, studies from our laboratory showed that excess thrombin resulted in the induced expression of VEGF following MPA treatment of human endometrial stromal cells (Lockwood et al., 2002).

We hypothesize that pathological angiogenesis and AUB following LTPOC is in part mediated by the interaction of TF-FVIIa resulting in intracellular signaling cascades or indirectly resulting in signaling via the production of thrombin.

Conclusions

For the sake of simplicity, the mechanisms proposed to account for the onset of AUB and enhanced microvessel fragility observed after LTPOC treatment have been treated as independent events. However, several of the pathways are likely to occur in concert and thus be interdependent. This could arise for related reasons, including reduced blood flow resulting in local hypoxia and reperfusion injury in turn creating an inflammatory, pro-thrombotic, pro-angiogenic environment. This could in turn create a feed-forward loop to further induce the aberrant expression and activation of molecules and pathways leading to fragile, distended vessels that are prone to bleed.

References

Archer D.F., Philput C.A. and Weber M.E. (1996). Management of irregular uterine bleeding and spotting associated with Norplant. Hum. Reprod. 2 (Suppl) 2, 24-30.

Bach R.R. (1988). Initiation of coagulation by tissue factor. CRC Crit. Rev. Biochem. 23, 339-368.

Belsey E.M. (1998). The association between vaginal bleeding patterns and reasons for discontinuation of contraceptive use. Contraception 38, 207-225.

Browder T., Folkman J. and Pirie-Shepherd S. (2000). The hemostatic system as a regulator of angiogenesis. J. Biol. Chem. 275, 1521-1524.

Charnock-Jones D.S., Macpherson A.M., Archer D.F., Leslie S., Makkink W.K. and Sharkey A.M. (2000). The effect of progestins on vascular endothelial growth factor, oestrogen receptor and progesterone receptor immunoreactivity and endothelial cell density in human endometrium. Hum. Reprod. 15, (suppl 3), 85-95.

Cheung A.H., Stewart R.J. and Marsden P.A. (1998). Endothelial Tie2/Tek ligands angiopoietin-1 (ANGPT1) and angiopoietin-2 (ANGPT2): regional localization of the human genes to 8q22.3-q23 and 8p23. Genomics 48, 89-91.

Classen-Linke I., Alfer J., Krusche C.A., Chwalisz K., Rath W. and Beier H.M. (2000). Progestins, progesterone receptor modulators, and progesterone antagonists change VEGF release of endometrial cells in culture. Steroids 6, 763-771.

Cui M.Z., Parry G.C.N., Oeth P., Larson H., Smith M., Huang R.P., Adamson E.D. and Mackman N. (1996). Transcriptional regulation of the tissue factor gene in human epithelial cells is mediated by SP1 and EGR-1. J. Biol. Chem. 271:2731-2739.

Davis S., Aldrich T.H., Jones P.F., Acheson A., Compton D.L., Jain V., Ryan T.E., Bruno J,. Radziejewski C., Maisonpierre P.C. and Yancopoulos G.D. (1996). Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell 87, 1161-1169.

Donovan-Peluso M., George L.D. and Hasset A.C. (1994). Lipopolysaccharide induction of tissue factor expression in THP-1 monocytic cells, protein-DNA interactions with the promoter. J. Biol. Chem. 269, 1361-1369.

Fan M. and Sujuan G. (1996). Menstrual bleeding patterns in Chinese women using the Norplant subdermal implant. Hum. Reprod. 2 Suppl 2, 14-19.

Ferrara N. (1999). Molecular and biological properties of vascular endothelial growth factor. J. Mol. Med. 77, 527-543.

Gale N.W. and Yancopoulos G.D. (1999). Growth factors acting via endothelial cell-specific receptor tyrosine kinases: VEGFs, angiopoietins, and ephrins in vascular development. Genes Dev. 13, 1055-1066.

Gale N., Thurston G., Hackett S., Renard R., Wang Q., McClain J., Martin C., Witte C., Witte M., Jackson D., Suri C., Campochiaro P., Wiegand S. and Yancopoulos G. (2002). Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by angiopoietin-1. Dev Cell. 3, 411-423.

Guha A., Bach R., Konigsberg W. and Nemerson Y. (1986). Affinity purification of human tissue factor: interaction of factor VII and tissue factor in detergent micelles. Proc. Natl. Acad. Sci. USA 83, 299-302.

Hague S., MacKenzie I.Z., Bicknell R. and Rees M.C. (2002). In-vivo angiogenesis and progestogens. Hum. Reprod. 17, 786-793.

Hickey M. and Fraser I.S. (2000). A functional model for progestogen-induced breakthrough bleeding. Hum. Reprod. 15 Suppl 3,1-6.

Hickey M., Fraser I., Dwarte D. and Graham S. (1996). Endometrial vasculature in NORPLANT users: preliminary results from a hysteroscopic study. Hum. Reprod. 2: Suppl 2, 35-44.

Hickey M., Simbar M., Markham R., Young L., Manconi F., Russell P. and Fraser I.S. (1999). Changes in vascular basement membrane in the endometrium of Norplant users. Hum. Reprod. 14, 716-721.

Hickey M., Carati C., Manconi F., Gannon B.J., Dwarte D. and Fraser I.S. (2000). The measurement of endometrial perfusion in Norplant users: a pilot study. Hum. Reprod. 15, 1086-1091.

Holash J., Wiegand S.J. and Yancopoulos G.D. (1999). New model of tumor angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF. Oncogene 18, 5356-5362.

Hornung D., Lebovic D.I., Shifren J.L., Vigne J.L. and Taylor R.N. (1988). Vectorial secretion of vascular endothelial growth factor by polarized human endometrial epithelial cells. Fertil Steril. 69, 909-915.

Krikun G., Critchley H., Schatz F., Wan L., Caze R., Baergen R.N. and Lockwood C.J. (2002). Abnormal uterine bleeding during progestin-only contraception may result from free radical-induced alterations in angiopoietin expression. Am. J. Pathol. 161, 79-86.

Krikun G., Schatz F-. ,Finlay T., Kadner S., Mesia A., Gerrets R. and Lockwood C.J. (2000). Expression of angiopoietin-2 by human endometrial endothelial cells: Regulation by hypoxia and inflammation. Biochem. Biophys. Res. Comm. 275, 59-163.

Lau T.M., Affandi B. and Rogers P.A. (1999). The effects of levonorgestrel implants on vascular endothelial growth factor expression in the endometrium. Mol. Hum. Reprod. 557-563.

Lebovic D.I,. Shifren J..L., Ryan I.P., Mueller M.D, Korn A.P, Darney P.D. and Taylor R.N. (2000). Ovarian steroid and cytokine modulation of human endometrial angiogenesis. Hum. Reprod. 15, Suppl 3, 67-77.

Lockwood C.J., Nemerson Y., Guller S., Krikun G., Alvarez M., Hausknecht V., Gurpide E. and Schatz F. (1993a). Progestational regulation of human endometrial stromal cell tissue factor expression during decidualization. J. Clin. Endocrinol. Metab. 7, 231-236.

Lockwood C.J., Nemerson Y., Krikun G., Hausknecht V., Markiewicz L., Alvarez M., Guller S. and Schatz F. (1993b). Steroid-modulated stromal cell tissue factor expression: a model for the regulation of endometrial hemostasis and menstruation. J. Clin. Endocrinol. Metab. 77, 1014-1019.

Lockwood C.J. and Schatz F. (1996). A biological model for the regulation of peri-implantational hemostasis and menstruation. J. Soc. Gynecol. Invest. 3,159-165.

Lockwood C.J., Krikun G., Runic R., Schwartz L.B., Mesia A.F. and Schatz F. (2000). The role of tissue factor in regulating endometrial haemostasis: implications for progestin-only contraception. Hum. Reprod. 15 (Suppl 3) 144-151.

Lockwood C.J., Krikun G. and Schatz F. (2001). Decidual cell-expressed tissue factor maintains hemostasis in human endometrium. Ann. NY Acad. Sci. 943, 77-88.

Lockwood C.J., Krikun C.J., Koo A.B., Kadner S. and Schatz F. (2002). Differential effects of thrombin and hypoxia on endometrial stromal and glandular epithelial cell vascular endothelial growth factor expression. J. Clin. Endocrinol. Metab. 87, 4280-4286.

Maisonpierre P.C., Suri C., Jones P.F., Bartunkova S., Wiegand S.J, Radziejewski C., Compton D., McClain J., Aldrich T.H., Papadopoulos N., Daly T.J., Davis S., Sato T.N. and Yancopoulos G.D. (1997). Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 277, 55-60.

Maragoudakis M.E., Tsopanoglou N.E and Andriopoulou P. (2002). Mechanism of thrombin-induced angiogenesis. Biochem. Soc. Trans. 30, 173-177.

Moll T., Czyz M., Holzmüller H., Hofer-Warbinek R., Wagner E., Winkler H., Bach F.H. amd Hofer E. (1995). Regulation of the tissue factor promoter in endothelial cells, binding of NFkB, AP-1 and SP1-like transcription factors. J. Biol. Chem. 270, 3849-3857.

Nemerson Y. (1988). Tissue factor and hemostasis. Blood 71, 1-8.

Oh H., Takagi H., Suzuma K., Otani A., Matsumura M. and Honda Y. (1999). Hypoxia and vascular endothelial growth factor selectively

up-regulate angiopoietin-2 in bovine microvascular endothelial cells. J. Biol .Chem. 274, 15732-15739.

O'Reilly F.M, Casper K.A., Otto K.B., Sexton S.A. and Swerlick R.A. (2003). Regulation of tissue factor in microvascular dermal endothelial cells. J. Invest. Dermatol. 120, 489-494

Pendurthi U.R. and Rao L.V. (2002). Factor VIIa/tissue factor-induced signaling: a link between clotting and disease. Vitam. Horm. 64, 323-355.

Peppelenbosch M.P. and Versteeg H.H. (2001). Cell biology of tissue factor, an unusual member of the cytokine receptor family. Trends. Cardiovasc. Med. 11, 335-339

Peters K.G., deVries C. and Williams L.T. (1993). Vascular endothelial growth factor receptor expression during embryogenesis and tissue repair suggests a role in endothelial differentiation and blood vessel growth. Proc. Natl. Acad. Sci. USA 90, 8915-8919.

Popovici R.M., Irwin J.C., Giaccia A.J. and Giudice L.C. (1999). Hypoxia and cAMP stimulate vascular endothelial growth factor (VEGF) in human endometrial stromal cells: potential relevance to menstruation and endometrial regeneration. J. Clin. Endocrinol. Metab. 84, 2245-2248.

Robinson C.J. and Stringer S.E. (2001). The splice variants of vascular endothelial growth factor (VEGF) and their receptors. J. Cell. Sci. 114, 853-865.

Rogers P.A.W. (1996). Endometrial vasculature in Norplant users. Hum. Reprod. 2 (Suppl 2) 45-50.

Rogers P.A., Plunkett D. and Affandi B. (2000). Perivascular smooth muscle alpha-actin is reduced in the endometrium of women with progestin-only contraceptive breakthrough bleeding. Hum. Reprod. 15 (Suppl 3) 78-84

Runic R., Schatz F., Krey L., Demopoulos R., Thung S., Wan L. and Lockwood C.J. (1997). Alterations in endometrial stromal cell tissue factor protein and messenger ribonucleic acid expression in patients experiencing abnormal uterine bleeding while using Norplant-2 contraception. J. Clin. Endocrinol. Metab. 82, 1983-1988.

Runic R., Schatz F., Wan L., Demopoulos R., Krikun G., and Lockwood C.J. (2000). Effects of norplant on endometrial tissue factor expression and blood vessel structure. J. Clin. Endocrinol. Metab. 85, 3853-3859.

Ruf W. and Mueller B.M. (1996). Tissue factor in cancer angiogenesis and metastasis. Curr. Opin. Hematol. 3, 379-384.

Senger D.R., Ledbetter S.R., Claffey K.P., Papadopoulos-Sergiou A., Peruzzi C.A. and Detmar M. (1996). Stimulation of endothelial cell migration by vascular permeability factor/vascular endothelial growth factor through cooperative mechanisms involving the alphavbeta3 integrin, osteopontin, and thrombin. Am. J. Pathol. 149, 293-305

Sharkey A.M., Day K., McPherson A., Malik S., Licence D., Smith S.K. and Charnock-Jones DS. (2000). Vascular endothelial growth factor expression in human endometrium is regulated by hypoxia. J. Clin. Endocrinol. Metab. 85, 402-409.

Shifren J..L., Tseng J.F., Zaloudek C.J., Ryan I.P., Meng Y.G., Ferrara N., Jaffe R.B. and Taylor R.N. (1996). Ovarian steroid regulation of vascular endothelial growth factor in the human endometrium: implications for angiogenesis during the menstrual cycle and in the pathogenesis of endometriosis. J. Clin. Endocrinol Metab. 81, 3112-3118.

Shoji M., Hancock W.W. and Abe K. (1998). Activation of coagulation and angiogenesis in cancer: immunohistochemcial localization in situ of clotting proteins and vascular endothelial growth factor in human cancer. Am. J. Pathol. 152, 399-411.

Smith S.K., Torry D.S., Holt V.J., Keenan J.A., Harris G., Caudle M.R. and Torry R.J. (1996). Vascular endothelial growth factor expression in cycling human endometrium. Fertil. Steril. 66, 72-80

Smith S.K. (1988). Angiogenesis, vascular endothelial growth factor and the endometrium. Hum. Reprod. Update 4, :509-519.

Stenina O.I. (2003). Regulation of gene expression in vascular cells by coagulation proteins. Curr. Drug. Targets 4, 143-158.

Su-Juan G., Ming-Kun D., Ling-De Z., Ying-Lin L, Shu-Hua W. and Sivin I. (1994). A 5-year evaluation of NORPLANT contraceptive implants in China. Obstet. Gynecol. 83, 673-678.

Suri C., Jones P.F., Patan S., Bartunkova S., Maisonpierre P.C., Davis S., Sato T.N and Yancopoulos G.D. (1996). Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell 87, 1171-1180.

Suri C., McClain J., Thurston G., McDonald D.M., Zhou H., Oldmixon E.H., Sato T.N. and Yancopoulos G.D. (1999). Increased vascularization in mice overexpressing angiopoietin-1. Science 282, 468-471.

Tischer E., Mitchell R., Hartman T., Silva M., Gospodarowicz D., Fiddes J.C. and Abraham J.A. (1991). The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. J. Biol. Chem. 266, 11947-11954.

Torry D.S., Holt V.J., Keenan J.A., Harris G., Caudle M.R. and Torry R.J. (1996). Vascular endothelial growth factor expression in cycling human endometrium. Fertil. Steril. 66, 72-80.

Torry D.S. and Torry R.J. (1997). Angiogenesis and the expression of vascular endothelial growth factor in endometrium and placenta. Am. J. Reproductiv. Immunol. 37, 21-29.

Tsopanoglou N.E. and Maragoudakis M.E. (1999). On the mechanism of thrombin-induced angiogenesis. Potentiation of vascular endothelial growth factor activity on endothelial cells by up-regulation of its receptors. J. Biol Chem. 274, 23969-23976.

Valenzuela D.M., Griffiths J.A., Rojas J., Aldrich T.H., Jones P.F., Zhou H., McClain J., Copeland N.G., Gilbert D.J., Jenkins N.A., Huang T., Papadopoulos N., Masonpierre P.C., Davis S. and Yancopoulos G.D. (1999). Angiopoietins 3 and 4: Diverging gene counterparts in mice and humans. Proc. Natl. Acad. Sci. USA 96, 1904-1909.

Versteeg H.H., Peppelenbosch M.P. and Spek C.A. (2001). The pleiotropic effects of tissue factor: a possible role for factor VIIa-induced intracellular signaling? Thromb. Haemost. 86, 1353-1359

Ward N.L and Dumont D.J. (2002). The angiopoietins and Tie2/Tek: adding to the complexity of cardiovascular development. Semin. Cell. Dev. Biol. 13, 19-27.

Yokoyama Y., Sato S., Futagami M., Fukushi Y., Sakamoto T., Umemoto M. and Saito Y. (2000). Prognostic significance of vascular endothelial growth factor and its receptors in endometrial carcinoma. Gynecol. Oncol. 77, 413-418.

Zhang L., Scott P.A., Turley H., Leek R., Lewis C.E., Gatter K.C, Harris A.L, Mackenzie I..Z, Rees M.C. and Bicknell R. (1998). Validation of anti-vascular endothelial growth factor (anti-VEGF) antibodies for immunohistochemical localization of VEGF in tissue sections: expression of VEGF in the human endometrium. J. Pathol. 185, 402-408.

Accepted July 10, 2003