VEGF, bFGF, and Their Receptors in the Endometrium of Rhesus Monkey During Menstrual Cycle and Early Pregnancy

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ABSTRACT A number of cytokines and growth factors are known to modulate proliferation and differentiation of human endometrium. In this study, the expression of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and VEGF receptors, fms-like tyrosine kinase (Flt1) and kinase insert domain-containing region (KDR), and bFGF receptor 1 (Flg) were examined in the endometrium of rhesus monkey on Day 5, 10, 16, 20, 25 of menstrual cycle and on Day 19 of early pregnancy. Western blot analysis showed the specificity of the anti-human antibodies with the monkey tissue. The expression of mRNA and protein of VEGF was correlated with that of its receptor KDR, which was detected in epithelial, vascular, and myometrial cells. The localization of bFGF and its receptor Flg was similar to that of VEGF, except that the Flg was absent in the endothelial cells. Strong expression of VEGF and bFGF in the glandular epithelial cells was observed in the proliferative phase, declined in the secretory phase during the cycle. Stronger staining of these factors was also observed in the decidual cells of the pregnant uterus, as compared with the stromal cells of cycling uterus. No expression of Flt1 was detected in the tissue examined in this study. These data suggest that VEGF, bFGF, and their receptors play important roles in epithelial and stromal development, angiogenesis, and blood vessel function in the endometrium during the menstrual cycle and early pregnancy of the rhesus monkey. *Mol. Reprod. Dev. 68: 456–462, 2004.*
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Key Words: VEGF; bFGF; endometrium; menstrual cycle; early pregnancy; rhesus monkey

INTRODUCTION

Endometrium undergoes cyclic proliferation and differentiation of both the glandular epithelial and the stromal cells for preparation of embryo implantation. Ovarian steroids are the prime modulators of these changes, they interact with local growth factors to regulate growth and differentiation of endometirium. Endometrial regeneration of preparation for a receptive endomerium involves apoptosis, proteolysis, and angiogenesis. In recent studies, we have reported the changes in the expression of molecules related to apoptosis and proteolysis in primate implantation site of early pregnancy (Feng et al., 2000, 2001; Gao et al., 2001a,b).

Angiogenesis is a fundamental process of generating new capillary blood vessels from preexisting blood vessels, it occurs in a variety of physiological and pathological settings. However, it rarely happens in the adult under normal circumstances, with the exception of the female reproductive organs, such as the corpus luteum and endometrium during menstrual cycle and pregnancy. Angiogenesis is crucial for the development of endometrium as well as for implantation and placentation (Popovici et al., 1999).

A number of cytokines and growth factors are known to modulate angiogenesis in human endometrium in vitro and in vivo with a paracrine action. Among these factors, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) appear to be potent angiogenesis inducers (Seghezzi et al., 1998). It has been suggested that these factors belong to one of the major families of heparin-binding angiogenic factors. This suggestion is supported by observations that expressions of FGF/FGF receptors and VEGF/VEGF receptors have been identified in the uterus of rodents, the pigs, the sheep, and the human during its growth, remodeling, and placentation. VEGF is a protein with angiogenic activity and a potent stimulator of microvascular permeability (Dvorak et al., 1995; Ferrara and Davis-Smyth, 1997). It plays an important role in physiological and pathological neovascularization via its receptors Flt1/VEGFR1 and kinase insert domain-containing region (KDR)/VEGFR2, both of which have tyrosine kinase activity (Klagsbrun and D'Amore, 1996). Four different splice variants of VEGF and their two receptors are known to be present in human endometrium (Moller et al., 2001). Previous studies have shown that

VEGF was expressed in the endometrium and may be involved in angiogenesis and vascular permeability necessary for successful implantation (Chakraborty et al., 1995). bFGF is the prototype member of a distinct class of heparin-binding growth factors and stimulates angiogenesis by binding to either of its two receptors, FGFR-1 (Flg) and FGFR-2, both of which have tyrosine kinase activity (Friesel et al., 1989). bFGF and its two receptors has been identified in rodent (Wordinger et al., 1992; Carlone and Rider, 1993), sheep (Reynolds et al., 1998), and primate endometrium (Samathanam et al., 1998), Flg seems to be the more important receptor in this tissue (Sangha et al., 1997). bFGF is a potent mitogen in vitro for different cell types, including vascular endothelial cells and figbroblasts. bFGF in cultured endothelial cells induced an angiogenic phenotype consisting of increase in the proliferation, migration, proteinase production, and expression of specific integrins. However, to our knowledge, the cellular source and expression pattern of VEGF, bFGF, and their receptors in the endometrium of primate during the menstrual cycle have not been defined consistently (Seghezzi et al., 1998). The aim of the present study, therefore, was to investigate the localization and possible role of VEGF, bFGF, and their receptors in the cyclic and pregnant endometrium of the rhesus monkey, which is similar to human in terms of reproduction (Yue et al., 2000).

MATERIALS AND METHODS
Animals

Healthy, adult male and female rhesus monkeys (*Macaca mulatta*) from the monkey colony of the Primate Research Center, Kunming Institute of Zoology, Chinese Academy of Sciences (CAS) were used. All experimental work was approved by the Animal Ethics Committees of both the Institute of Zoology and the Kunming Institute Primate Research Center, CAS. The animals were caged individually and were evaluated daily by visual examination of the perineum for menses, with the onset of menses defined as Day 1 of the menstrual cycle (MD1). Adult female monkeys with regular menstrual cycles of approximately 28 days were chosen for this study. Male rhesus monkeys of proven fertility from previous mating were used for mating. Female monkeys on Day 11 of their menstrual cycle were caged with a male monkey for 3 days. Vaginal smears were examined the next morning for the presence of sperm. The day a positive smear for sperm was designated as Day 1 of pregnancy. The presence of a conceptus was confirmed by ultrasound examination. The monkeys were sacrificed (3 animals each group), the uteri were removed and cut to pieces at MD5, MD10, MD16, MD20, MD25, and on Day 19 of pregnancy, respectively, and the specimens was quickly washed in cold phosphate-buffered saline (PBS) to remove adherent blood, then placed in cold 4% paraformaldehyde fixative for 24 hr at 4°C and further processed through graded dehydration, clearing, and embedding in paraf-fin for immunohistochemistry and in situ hybridization. Part of specimens were cryopreseved at −70°C for Western blot.

Reagents

Primary antibodies including rabbit anti-human bFGF (SC-79), rabbit anti-human Flg (SC-276), mouse anti-human VEGF (SC-7269), rabbit anti-human Flt1 (SC-316), mouse anti-human KDR (SC-6251) were obtained from Santa-Cruz (Santa Cruz, CA). Biotin labeled secondary antibodies, AP-conjugated avidin, and Vector-red substrates were from Vector (Vector Laboratories, Burlingame, CA). Digxigenenin (DIG)-RNA labeling kit, blocking reagent, alkaline phosphate conjugated anti-DIG antibody, 5-bromo-4-chloro-3-indoxyl phosphate/nitro-blue tetrazolium chloride (BCIP/NBT) were purchased from Roche (Mannheim, Germany). Proteinase K was purchased from Merck-Schuchardt (Darmstadt, Germany). Restriction enzyme and T-vector were purchased from Promega Co., Ltd., (Madison, WI). Ficoll, levamisole, and dextrasulphate were purchased from Sigma (St Louis, MO). Super-Signal® West Pico substrate was from PIERCE (Rockford, IL).

Western Blot

Immunoblot was done as previously described (Zhou et al., 2002) to verify the specificity of the antibodies used in this study with the monkey endometrium at Day 19 of pregnancy. The tissue was homogenized and the supernatant from ultracentrifugation was run on a 10% SDS–PAGE gel under reduced conditions. After transfer to the polyvinylidene difluoride membrane, individual lanes were cut and blocked with 5% nonfat milk/PBS for 1 hr, followed by incubation at 20°C for 1 hr with primary antibody (IgG, 0.2 μg/ml) in 5% milk/PBS. The membranes were washed three-times for 5 min each in 5% milk/PBS and incubated with HRP-conjugated horse anti-mouse IgG (0.2 μg/ml) in 5% milk/PBS for 1 hr, respectively. The membranes were washed in PBS three-times for 5 min each, followed by 10 min of incubation with SuperSignal® West Pico substrate, then exposed on X-ray film. Primary antibodies were replaced with the same concentration and the same origin of normal IgG as a negative control.

In Situ Hybridization

The following probes were used: a 450 bp fragment of human bFGF and a 405 bp fragment of human VEGF were coloned into T-vector, respectively, based on the reports of cDNA sequences. Using linearized templates, single stranded sense, and antisense RNA probes were transcribed using a digoxigenin RNA labeling kit and using the protocol supplied by the manufacturer.

The in situ hybridization protocol used in the present study was based on the previously described methods (Gao et al., 2001b) with slight change, and is briefly described below. Sections (5 μm) of formalin-fixed, paraffin-embedded tissue were de-paraffinized, rehybridited, and digested with proteinase K (10 μg/ml) for

30 min at 37°C and post-fixed in 4% paraformaldehyde at 4°C for 10 min. The tissue sections were prehybridized for 2 hr at 48°C in 4 × SSC (1 × SSC: 150 mmol/L NaCl, 15 mmol/L tri-sodium citrate, pH 7.0)/50% formamide, followed by overnight hybridization at 48°C in 50 µl hybridization buffer consisting of 20–30 ng of dig-labeled RNA probe, 50% formamide, 10% dextran sulphate, 1 × Denhardt's solution, 10 mmol/L dithiothreitol, 1 mg/ml yeast-RNA, 2 × SSC, 10 mM Tris-HCl, and 0.5% sodium dodccyl sulfate. The sections were then washed with 2 × SSC, 1 × SSC both for 15 min at 42°C. This was followed by treatment with RNase A (20 µg/ml) for 30 min at 37°C. The sections were sequentially washed twice each with 1 × SSC followed by 0.1 × SSC for 30 min at 48°C. Following washing with buffer 1(0.1 mol/L Tris, 150 mmol/L NaCl, pH 7.5) for 10 min at room temperature, blocking solution was applied containing 0.1% Triton X-100, 1% blocking reagent in buffer I for 1 hr anti-dig-alkaline phophatase (1:500) in blocking solution was then applied overnight at 4°C after which the color reaction was developed with BCIP/NBT solution for 2 hr in the dark at room temperature. Sections hybridized with the corresponding sense probes served as negative controls.

Immunohistochemistry

Serial 4 µm sections of tissue were cut, deparaffinized, and rehydrated through degraded ethanol. Antigen retrieval was performed by incubating the sections in 0.01 mol citrate buffer (pH 6.0) at 98°C for 20 min (for bFGF, Flg, and KDR) or in 0.1 mol/L EDTA solution (pH 8.5) at 94°C for 15 min (for VEGF) and cooling at room temperature for 20 min. Non-specific binding was blocked with 10% (v/v) normal goat serum in PBS for 1 hr. The sections were incubated with rabbit anti-human bFGF (1 µg/ml), rabbit anti-human flg (1 µg/ml), mouse anti-human VEGF (0.5 µg/ml), rabbit anti-human Flt1 (2 µg/ml), mouse anti-human KDR (0.2 µg/ml), respectively, in 10% goat serum for 2 hr. The sections were then incubated with biotinylated secondary antibody followed by an avidin-alkaline phophatase complex and the standard substrates (337.5 NBT and 175 µg/ml BCIP) or Vector-red substrates, according to the manufacturer's protocol. Endogenous alkaline phosphatase activity was inhibited by supplement 1 mmol/L levamisole into substrate. Primary antibodies were replaced with the same concentration and the same origin normal IgG as a negative control.

Microscopic Assessment

Endometrial tissues from three individual monkeys for each group were analyzed. Experiments were repeated at least three-times, and one representative from at least three similar results was presented. The mounted sections were examined using a Nikon microscope. For assessment of staining in cells of different compartments, semi-quantitative subjective scoring was done blinded by three investigators using a 4-scale system: "−" = nil; "+/−" = weak; "+" = moderate; and "++" = strong, as described by Yue et al. (2000).

RESULTS
Western Blot

In order to confirm the specificities of the antibodies used in this study and to provide the first line of evidences for the expression of VEGF, bFGF, and their receptors in the endometrium of rhesus monkey, we performed Western blot analysis on the tissue of Day 19 of pregnancy. As shown in Figure 1, the anti-VEGF antibody recognized bands corresponding to the three types of VEGF: $VEGF_{121}$, $VEGF_{165}$, and $VEGF_{189}$; anti-KDR binding a band of about 200 kDa; anti-bFGF antibody binding a band of about 18 kDa and anti-Flg antibody binding a band of about 150 kDa, respectively. There was no obvious band could be detected in all negative control studies (data not shown).

Immunohistochemistry

VEGF immunohistochemistry. During the menstrual cycle, staining of VEGF was mainly observed in luminal epithelial cells, glandular epithelial cells (Fig. 2A), some immunocyte-like cells (Fig. 2G), the vascular endothelial cells (Fig. 2H,I) and the smooth muscle cells around the mid and large sized vessels (Fig. 2I). The staining in the epithelial cells was much stronger on MD5, MD10, and MD16 than that on MD20 and MD25. Weak VEGF staining was also detected in the stromal cells during the menstrual cycle and the staining appeared to be unchanged through out the cycle. However, it was significantly increased in the decidualized stromal cells (Fig. 2F). A weak VEGF staining was also detected in myometrium (Fig. 2J), and the intensity was not changed either through out the menstrual cycle or by pregnancy.

Flt1 immunohistochemistry. Flt1 immunostaining of vascular endothelial cells, glandualar epithelial cells, and stromal cells was not observed or negligible during the entire menstrual cycle (data not shown).

KDR immunohistochemistry. Similar to the expression pattern of VEGF, strong KDR immunostaining was also observed in the glandular epithelial cells at different stages (Fig. 3A–F), in the vascular smooth muscle cells (Fig. 3G), glandular epithelial cells
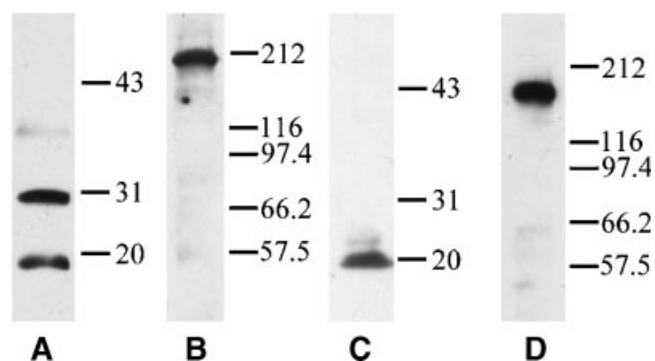

Fig. 1. Western blot analysis of (**A**) vascular endothelial growth factor (VEGF), (**B**) kinase insert domain-containing region (KDR), (**C**) basic fibroblast growth factor (bFGF), and (**D**) Flg in the rhesus monkey endometrium on Day 19 of pregnancy.
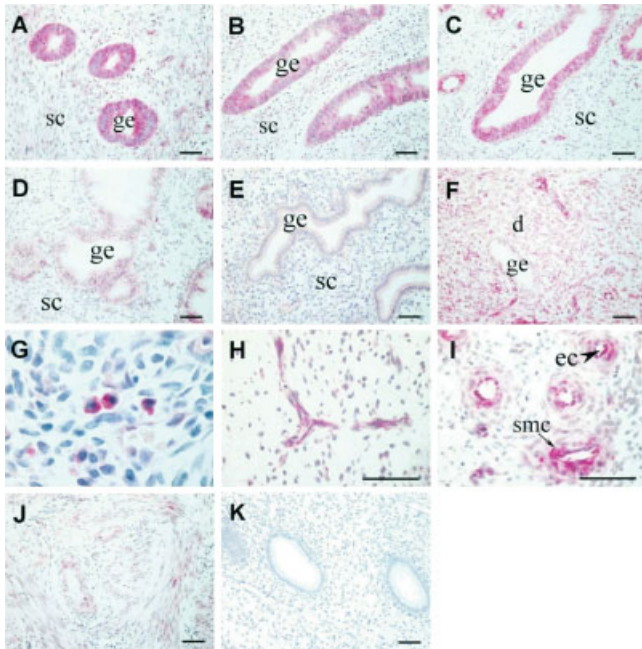
Fig. 2. Immunohistochemical staining of VEGF in the endometrium of rhesus monkey. Positive staining is red, and nuclear counterstain blue. **A**: Menstrual cycle Day (MD) 5; (**B**) MD10; (**C**) MD16; (**D**) MD20; (**E**) MD25; (**F**) D19 of early pregnancy. **G**: Immunocyte-like cells in endometrium on MD5. **H**: Capillary blood vessels in endometrium on MD10. **I**: Arteries in the endometrium of pregnant tissue with arrowhead indicating endothelial cells and arrow indicating smooth muscle cells. **J**: Myometrium. **K**: Negative control on MD16. ge, glandular epithelium; sc, stromal cells; ec, endothelial cells; smc, smooth muscle cells; d, decidual cells. Scale bars represent 50 μm.
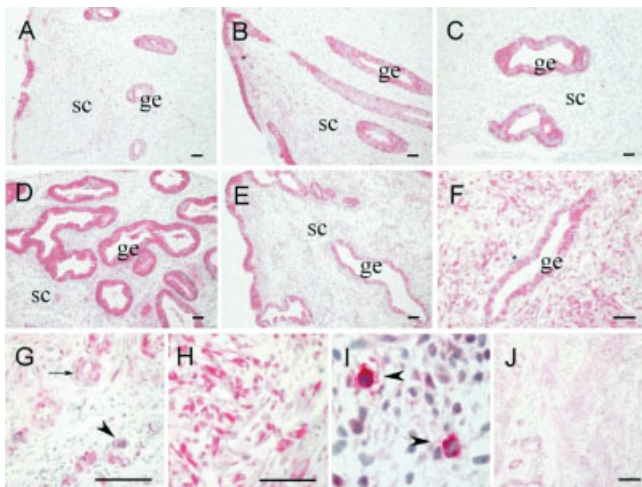

Fig. 3. Immunohistochemical staining of KDR in the endometrium of rhesus monkey. Positive staining is red, and nuclear counterstain blue. **A**: MD5; (**B**) MD10; (**C**) MD16; (**D**) MD20; (**E**) MD25; (**F**) D19 of early pregnancy. **G**: Microvessels (indicated by arrowhead) and arteries (indicated by arrow) in the endometrium on MD10. **H**: Decidual cells in pregnant endometrium. **I**: Immunocyte-like cells in endometrium on MD5. **J**: Myometrium. ge, glandular epithelium; sc, stromal cells. Scale bars represent 50 μm.
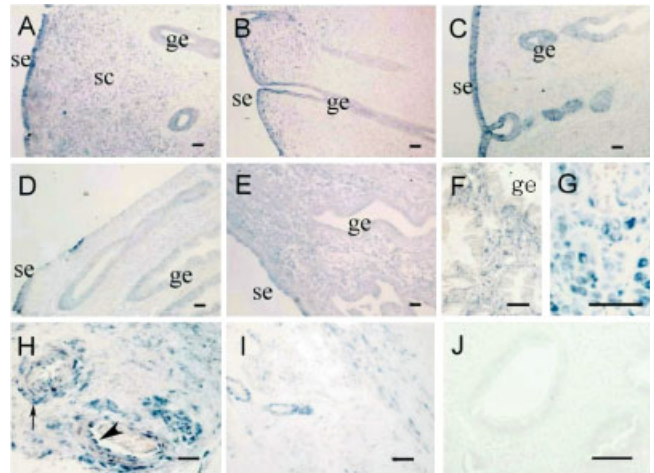

Fig. 4. Immunohistochemical staining of bFGF in the endometrium of rhesus monkey. **A**: MD5; (**B**) MD10; (**C**) MD16; (**D**) MD20; (**E**) MD25; (**F**) D19 of early pregnancy. **G**: Decidual cells in pregnant endometrium. **H**: Spiral arteries in the endometrium of pregnant uterus with arrowhead indicating endothelial cells and arrow indicating smooth muscle cells. **I**: Myometrium. **J**: Negative control on MD16. ge, glandular epithelium; se, surface epithelium; sc, stromal cells. Scale bars represent 50 μm.

(Fig. 3A–F), and in some immunocyte-like cells (Fig. 3I). It seemed the staining in epithelial cells on MD20 was stronger than that on the other days. The KDR-positive immunocyte-like cells presented mainly in the endometrium of the proliferative phase (data not shown). Again, an unchanged weak immunostaining was observed in the stroma during the cycle (Fig. 3A–E), and the staining remarkably increased in the decidual cells of early pregnancy (Fig. 3H).

bFGF immunohistochemistry. bFGF was present in both the glandular epithelium (ge), and the surface epithelium (se) on different days of the menstrual cycle
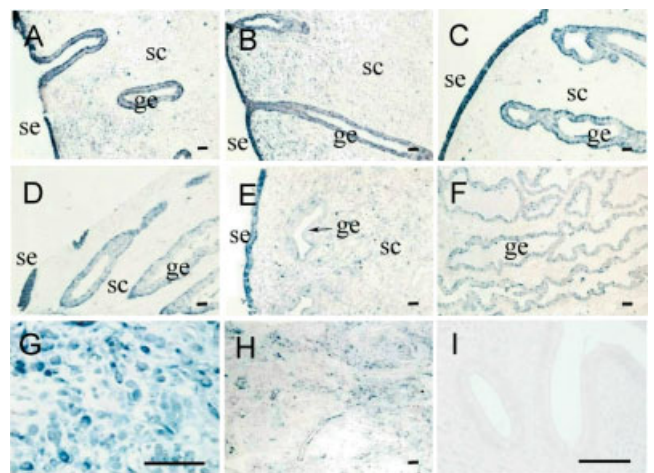

Fig. 5. Immunohistochemical staining of Flg in the endometrium of rhesus monkey. **A**: MD5; (**B**) MD10; (**C**) MD16; (**D**) MD20; (**E**) MD25; (**F**) D19 of early pregnancy. **G**: Decidual cells in pregnant endometrium. **H**: Myometrium. **I**: Negative control on MD16. ge, glandular epithelium; se, surface epithelium; sc, stromal cells. Scale bars represent 50 μm.

(Fig. 4A–E). It was also highly expressed in the endo-thelial cells (Fig. 4H) and less abundantly in the endometrial stroma and myometrial smooth muscle cells (Fig. 4I). A decrease in the glandular epithelial expression was observed on MD20 (Fig. 4D) and MD25 (Fig. 4E) of the menstrual cycle. Higher staining of bFGF was detected in the surface epithelium within the endometrium from MD5 to MD20. The signal in the stroma slightly decreased from MD16 to MD25 as compared with that on MD5 and MD10. No significant difference was found among the other cellular types. In the endometrium of pregnancy, a strong immunostaiining was observed in the dicidulized stromal cells on Day19 of pregnancy (Fig. 4G), but not in the epithelial cells (Fig. 4F, ge).

Flg immunohistochemistry. The Flg immunostaining pattern (Fig. 5) was similar to that of bFGF, but the staining in endothelial cells was not observed or negligible during the entire menstrual cycle and in early pregnancy.

The expression levels of VEGF, KDR, Flt1, bFGF, and Flg detected using immunohistochemistry were semi-quantitative evaluated and summarized in Table 1.

In Situ Hybridization

The expression of VEGF and bFGF was further investigated at the mRNA level using in situ hybridization technique. The mRNA expression VEGF (Fig. 6) and bFGF (Fig. 7) matched the protein expression detected in immunohistochemistry: The cellular localization of VEGF mRNA was found in the epithelial (Fig. 6A–F), endothelial (Fig. 6I, arrowhead), decidual (Fig. 6G), and some immunocyte-like cells (Fig. 6H, arrowhead). A strong epithelial signal for VEGF mRNA was detected in the tissues of MD5, 10, and 16. The bFGF mRNA was found in the epithelial cells (Fig. 7A–E) and blood vessels (Fig. 7G,H). A higher epithelial staining for bFGF mRNA was observed from MD5 to MD16.

DISCUSSION

The studies on VEGF and bFGF expression in human endometrium during the menstrual cycle have not given consistent results (Seghezzi et al., 1998). Therefore, Rhesus monkey that shares a very similar reproduction physiology with human was used for this study. Five time points (D5, D10, D16, D20, D25) in the menstrual cycle and one (D19) in the early pregnancy were examined by using immunohistochemistry and in situ hybridization. Western blot analysis was first performed to demonstrate that the anti-human primary antibodies were able to specifically recognize the corresponding monkey antigens.

In the present study, a menstrual cycle-dependent variation in VEGF was observed in the epithelium, endothelial cells and some scattered immunocyte-like stromal cells. A higher level of VEGF staining was present in the endometrium of proliferative and early secretory phase of the menstrual cycle. This is in agreement with the reports in human (Torry et al., 1996; Naresh et al., 1999) and suggests a role of VEGF as a mediator of the cyclic growth of blood vessels in the female reproductive tract. The phenomenon that VEGF expression in the human uterus was induced by estrogen in vitro (Charnock-Jones et al., 1993; Hyder and Stancel, 2000) indicates that the production of VEGF may be regulated by estrogen in vivo. Our results showed that VEGF expression in the epithelial and endothelial cells was coincident with the high level maternal estrogen phase. In stromal cells, low and constant staining for VEGF was observed during the whole cycle, whereas a stronger staining was found in the decidualized stromal cells of the pregnant uterus. These findings are consistent with the previous reports showing that VEGF expression was up-regulated by oestrogen and progesterone in endometrial stromal cells accompanied by decidualization (Chakraborty et al., 1995).

TABLE 1. Summary of the Immunohistochemical Experiments Identifying VEGF, Flt1, and KDR in Rhesus Monkey Uterus

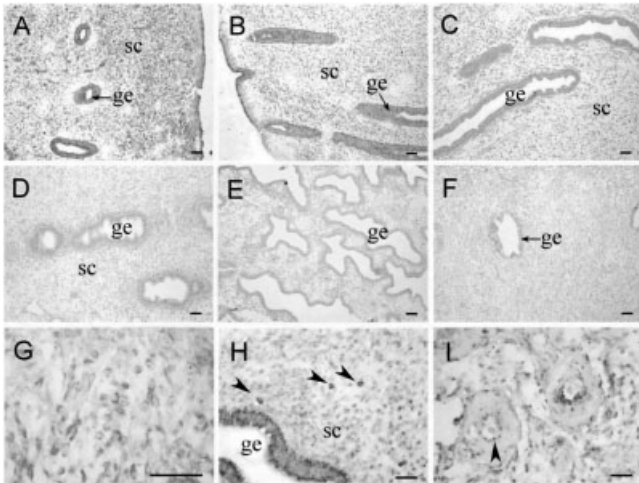|	MD5	MD10	MD16	MD20	MD25	Pregnancy D19
Endothelial cells						
VEGF	++	++	+	+/−	+/−	++
Flt1	−	−	−	−	−	−
KDR	+	+	+	+	+	+
bFGF	++	++	++	++	++	++
Flg	−	−	−	−	−	−
Stroma						
VEGF	+/−	+/−	+/−	+/−	+/−	+
Flt1	−	−	−	−	−	−
KDR	+/−	+/−	+/−	+/−	+/−	+++
bFGF	+/−	+/−	−	−	+/−	++
Flg	+/−	+/−	−	−	+/−	++
Epithelium						
VEGF	++	++	++	+	+/−	+/−
Flt1	−	−	−	−	−	−
KDR	++	++	++	+++	++	+
bFGF	+	+	++	+	+/−	+/−
Flg	++	++	++	+	+/−	+

MD, menstrual cycle Day.

Fig. 6. In situ hybridization of VEGF in the endometrium of rhesus monkey. **A**: MD5; (**B**) MD10; (**C**) MD16; (**D**) MD20; (**E**) MD25; (**F**) Day 19 of early pregnancy. **G**: Decidual cells in pregnant endometrium. **H**: Immunocyte-like cells in the endometrium on MD5 (indicated by arrowhead). **I**: Spiral arteries in the endometrium of pregnanct tissue with arrowhead indicating endothelial cells. ge, glandular epithelium; se, surface epithelium; sc, stromal cells. Scale bars represent 50 μm.

It is known that the mitogenic activity of VEGF is mediated mainly by the KDR receptor (Waltenberger et al., 1994; Cunningham et al., 1999). As the gene promoter area of human KDR contains the binding sites for glucocorticoid receptor/progesterone receptor (GR/PR) (Patterson et al., 1995), KDR appears to be under the control of progesterone. It was also reported that VEGF and VEGF receptor expression might be modified by factors such as cytokines (Patterson et al., 1996) and hypoxia (Sharkey et al., 2000). Our observation of higher

epithelial KDR expression presented in the endometrium of MD20 was similar to the reports by Moller et al. (2001). The obvious increase in KDR and VEGF in the decidual cells and the blood vessels of the implantation site suggests their important roles during implantation and pregnancy. It is well known that in addition to inducing endothelial cell proliferation, VEGF may mediate the increase in vascular permeability and vasodilatation (Torry et al., 1996), and provide trophic signals to the implanting embryo (Krussel et al., 1999) in the endometrium.

FGFs have potent pro-proliferative and angiogenic activities and hence might be involved in the tissue remodeling and neovascularization during the menstrual cycle and trophoblast invasion (Reynolds et al., 1998). In the present study, a higher epithelial bFGF and Flg level in the proliferative phase suggests a role of bFGF in the regulation of epithlial development. Presta (1988); Fujimoto et al. (1996); and Rider et al. (1997) demonstrated that the expression of bFGF was up-regulated by estrogen, and may play a role in blastocyst implantation (Sangha et al., 1997). This suggestion was supported by the fact that pregnancy was inhibited in rodents immunized against bFGF (Buscaglia et al., 1991). bFGF lacks a secretory signal sequence, therefore, endometrial neovascularization might be partially regulated by bFGF from surrounding cells, such as stromal or vascular smooth muscle cells (Reynolds et al., 1998). The high expression of bFGF/Flg in the decidua of rhesus monkey implies a role in controlling both stromal cell proliferation/differentiation and angiogenesis. The FGF receptor, Flg was not expressed in blood vessels during the menstrual cycle suggesting that Flg might be less important for the regulation of angiogenesis and may be critical for normal endometrial "maturation" and regeneration (Sangha et al., 1997).

A synergistic effect between the two growth factors on proliferation of endothelial cells and induction of angiogenesis has been demonstrated in vitro (Pepper et al., 1992; Goto et al., 1993; Zheng et al., 1999). In the present study, we described the co-localization of VEGF/KDR, and bFGF/Flg in the endometrium of rhesus monkey. However, a potential in vivo synergistic action of the two factors on primate endometrium remains to be studied.

CONCLUSIONS

The different expression patterns and regulation of VEGF/KDR and bFGF/Flg in the endometrium of rhesus monkey suggest that these cytokines may play different roles in the endometrial remodeling, proliferation and angiogenesis during menstrual cycle and early pregnancy under the regulation of sex steroids.

Fig. 7. In situ hybridization of bFGF in the endometrium of rhesus monkey. Positive staining is blue. **A**: MD5; (**B**) MD10; (**C**) MD16; (**D**) MD20; (**E**) MD25; (**F**) D19 of early pregnancy. **G**: Small blood vessels in the endometrium on MD10. **H**: Arteries in the endometrium of pregnanct uterus. **I**: Negative control on MD16. ge, glandular epithelium; se, surface epithelium; sc, stromal cells. Scale bars represent 50 μm.

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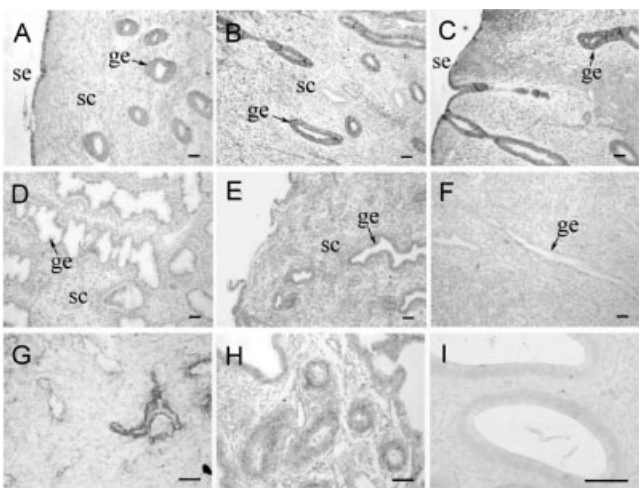
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