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VEGF, VEGFR-1 and VEGFR-2 immunoreactivity in the porcine arteries of vascular subovarian plexus (VSP) during the estrous cycle

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Abstract: Vascular endothelial growth factor (VEGF) is an important angiogenic factor in the female reproductive tract. It binds to cell surface through ligand-stimulatable tyrosine kinase receptors, the most important being VEGFR-1 (flt-1) and VEGFR-2 (flk-1). The broad ligament of the uterus is a dynamic organ consisting of specialized complexes of blood vessels connected functionally to the uterus, oviduct and ovary. Endothelial cells form an inner coating of the vessel walls and thus they stay under the influence of various modulators circulating in blood including ovarian steriods involved in developmental changes in the female reproductive system. The aim of the present study was to immunolocalize VEGF and its two receptors: VEGFR-1 and VEGFR-2 in the broad ligament of the uterus in the area of vascular subovarian plexus during different phases of the estrous cycle in pig and to determine the correlation between immunoreactivity of the investigated factors and phases of the estrous cycle. The study was performed on cryostat sections of vascular subovarian plexus stained immunohistochemically by ABC method. Specific polyclonal antibodies: anti-VEGF, anti-VEGFR-1 and anti-VEGFR-2 were used. Data were subjected to one-way analysis of variance. Our study revealed the presence of VEGF and its receptors in endothelial and smooth muscle cells of VSP arteries. All agents displayed phase-related differences in immunoreactivity suggesting the modulatory effect of VEGF, VEGFR-1 and VEGFR-2 on the arteries of the VSP in the porcine broad ligament of the uterus. (www.cm-uj.krakow.pl/FHC)

Key words: VEGF - VEGFR-1 - VEGFR-2 - VSP - Estrous cycle - Pig

Introduction

Since VEGF was first discovered in 1983 by Harold F. Dvorak and coworkers, a great majority of investigations have been conducted and many studies have described the role of this factor, its localization, modulatory effects and influence of other angiogenic and non-angiogenic factors as well as hormones that may regulate its expression [9, 15, 24, 25, 32, 34, 46, 52, 54, 56, 57, 58].

VEGF in the reproductive tract was first described in the uterus of the mouse, then human, rat, ewe, rabbit and monkey [6, 10 , 14, 41]. Vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) is an endothelial-cell-specific mitogen and thus, a prime regulator of vascular formation and function promoting migration of ECs (endothelial cells) and the formation of new vessels, regulating their permeability, inducing vascular leakage and fenestrations in ECs of small venules and capillaries [6, 44]. It is a basic, heparin-binding, homodimeric glycoprotein of 45 kD secreted by various tissues and cells such as: macrophages, glandular, epithelial, stromal, glial, tumor, smooth muscle cells, keratinocytes and ovarian cysts [47]. VEGF also regulates vascular tone, production of vasoactive molecules: von Willebrand factor, nitric oxide, cytokines [5, 24, 33, 49] and recruits progenitor endothelial cells from the bone marrow [8]. It has been found to be a key regulator of angiogenesis and vasculogenesis during early embryonic development [4, 20] and in early postnatal life [13].

Production of VEGF is regulated by several mechanisms: hypoxia [15, 50, 52], PDGF, EGF, TGF-β, steroid hormones, deregulated glucose values [53], cell differentiation, oncogenes [46] but all of these need further detailed investigations.

The VEGF family consists of several members: VEGF-A, VEGF-B, VEGF-C, VEGF-D, PLGF -

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placental growth factors and VEGF-E [22, 26, 29, 31, 38]. VEGF-A is best known and described as a major regulator of normal and abnormal angiogenesis [11, 12]. Several isoforms of VEGF were discovered. Initially, there were four variants. They consisted of 121, 165, 189 and 206 amino acids of almost indentical biological activities [16]. Nowadays, it is proven that more isoforms are generated including VEGF 183 [21] and VEGF 145 [40].

VEGF is a multifunctional factor. Its biological effects are mediated by two main tyrosine kinase receptors: VEGFR-1 and VEGRF-2 expressing different roles *in vivo* and thus responsible for functional variety of VEGF activities [8, 43, 45, 47, 57, 58]. Both receptors after ligand-induced dimerization activate intracellular signaling casades leading to their autophosphorylation [55, 56]. Activated receptor kinase answers in a biochemical and physiological fashion to stimulate DNA synthesis and determine the cellular response. However, VEGFR-2 reveals strong ligand-dependent tyrosine phosphorylation, whereas the response of VEGFR-1 is weak [56, 59].

Vascular endothelial growth factor is expressed at high levels in various normal male and female tissues [2, 36] as a regulatory factor participating in mammalian reproduction. Both, VEGF and its receptors are suggested to be influenced by ovarian steroids involved in developmental changes and functional activity in female reproductive tract [7, 14, 18, 19, 30, 32, 37, 51].

To gain a better understanding of the VEGF and its receptors expression in the broad ligament of the uterus - dynamic tissue, responding to steroid hormones and locally produced growth factors and cytokines throughout the cycle - our study was aimed at the detailed cellular localization of these agents.

This study is based on the hypothesis that during a normal reproductive cycle, levels of VEGF and its receptors in the endothelium and smooth muscle cells of VSP arteries might fluctuate and might be dependent on different stages of the cycle. Current studies characterized VEGF in terms of its role in vascular biology but many conclusions remain unclear with regard to hormonal regulation. Thus, it seemed to be important to examine VEGF and its receptors localization and expression in the area of dynamic hormonal influence - VSP of the broad ligament of the uterus.

Materials and methods

Animals and tissue preparation. The study was performed on 16 cyclic pigs in the following phases of the estrous cycle: follicular (17-21) n=4, early luteal (1-5) n=4, mid luteal (6-12) n=4 and late luteal (13-16) n=4. Tissues of vascular subovarian plexus (VSP) were excised below the ovary and then fixed in 4% paraformaldehyde (PFA) in 0.1M phosphate buffer (PB, pH=7.4). After several washes in 0.1M PB, all tissue samples were stored in 18% sucrose for cryoprotection. Ten μm cryostat sections were mounted on gelatin/chrome-alum-coated slides. Next, sections were stained immunohistochemically for VEGF, VEGFR-1 and VEGFR-2 activity.

Immunostaining for VEGF, VEGFR-1 and VEGFR-2. Immunostaining was done on consecutive sections (3 per uterus). In order to block endogenous peroxidase, sections were treated with hydrogen peroxide in methanol, washed in 0.1M PBS (phosphate buffer saline, pH=7.4), treated with glycine to block the activity of aldehyde groups and then blocked with 10% normal goat serum (NGS) for 1 h at room temperature. Next, sections were incubated overnight at room temperature with primary rabbit polyclonal antibodies: anti-VEGF (A:20, sc-152) in dilution 1:200, anti-VEGFR-1 (c-17, sc-316) in dilution 1:150, anti-VEGFR-2 (N-931, sc-505) in dilution 1:100 (Santa Cruz Biotechnology, Inc.). After overnight incubation with these antibodies, sections were washed in PBS and incubated again with anti-rabbit biotynylated IgG (Vectastatin ABC Kit) diluted 1:400 for 1 h. The following incubation with avidin-biotin-peroxidase complex (ABC Reagent, Vectastatin Kit) lasted 45 min at room temperature. The reaction was visualized by incubating sections in 0.3 mg/ml 3,3‘-diaminobenzidine tetrahydrochloride (DAB) and 0.01% hydrogen peroxide in PBS. Finally, sections were dehydrated and cover-slipped with DePeX. Negative controls were incubated with 10% NGS instead of the primary, secondary or both antibodies.

The intensity of immunostaining was estimated by measuring the optical density using Olympus DP Soft. Optical density is the method of measuring the intensity of visible products of tissue staining. The measured values range from 0 to 250 where 0 means black and 250 white colour. When the of immunostaining is strong the values are close to 0. However, all data in this study are presented as absolute values, so the stronger is tissue reaction the higher is the value of optical density. 20 pixel values were measured in the endothelium and muscular layer of each from 21 chosen arteries (7 from each section). That gave 1680 measurements per phase (420 measurements/uterus) for endothelium and the same for muscular layer. The data was subjected to one-way analysis of variance (Statistica 5,0). Differences among phases were tested by Tukey‘s test. All values were expressed as means (±SEM).

Results

Light microscopic analysis of the sections revealed estrous phase-related differences in expression of VEGF, VEGFR-1 and VEGFR-2 through the examined stages of the estrous cycle. The positive immunostaining was found in endothelial and smooth muscle cells of the arteries in the area of VSP.

Endothelial cells

Quantitative evaluation of the optical density of the VEGF, VEGFR-1 and VEGFR-2 immunoreactivity revealed their significant variability depending on the estrous cycle. The highest immunoreactivity of VEGF was displayed during the follicular phase of the estrous cycle (Figs. 1A, 2A) ($P \leq 0.001$). The intensity of staining decreased significantly during early luteal phase (Figs. 1B, 2A) to increase again during mid luteal phase (Figs. 1C, 2A). The weakest staining for VEGF was detected during early luteal phase and it did not significantly differ from the VEGF expression during late luteal phase (Figs. 1D, 2A) of the estrous cycle.

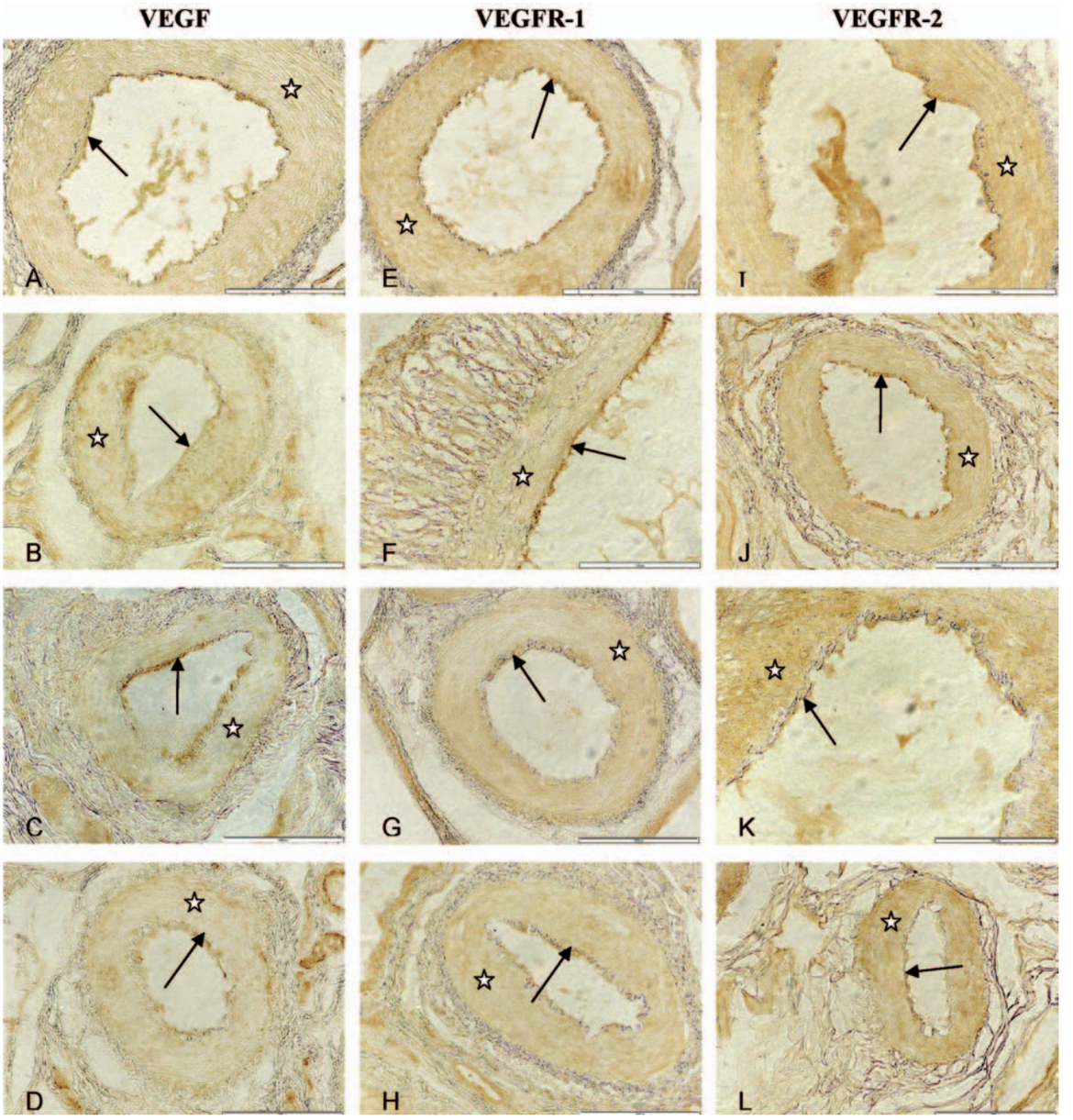

Fig. 1. Representative micrographs presenting the VEGF, VEGFR-1 and VEGFR-2 immunolocalization in the endothelial cells (arrowheads) and in the muscular layer (asterisks) of arterial vessels in the area of vascular subovarian plexus (VSP) of uterine broad ligament during the follicular (**A, E, I**), early luteal (**B, F, J**), mid luteal (**C, G, K**) and late luteal (**D, H, L**) phases of the estrous cycle in pig. Scale bar: 100 µm.

Comparing results for VEGF with those obtained for VEGFR-1, we found that the receptor's signal during the follicular phase was the most intense (Figs. 1E, 3A) and at the similar level as VEGF. In contrast to VEGF, VEGFR-1 displayed strong immunoreactivity in the early luteal phase (Figs. 1F, 3A). In the mid luteal phase (Figs. 1G, 3A), the immunostaining was the weakest.

Figures 1I-L present the immunoreactivity of VEGFR-2 in arterial endothelial cells. The strongest immunostaining was observed during the follicular phase of the estrous cycle (Figs. 1I, 4A), similarly to VEGF and VEGFR-1, although the differences between all phases were generally weak and displayed less significance.

Muscular layer

Immunostaining for all the examined factors in the muscular layer of VSP arteries was definitely weaker but optically visible through all the studied stages. VEGF displayed the strongest immunoreactivity during the follicular phase (Figs. 1A, 2B) and it was significantly different from other stages of the estrous cycle. The weakest immunoreaction for VEGF was observed during early luteal phase (Figs. 1B, 2B). This result corresponds with VEGFR-1 immunoreactivity because this receptor showed also the weakest reaction during early luteal phase (Figs. 1F, 3B), whereas the strongest immunostaining was displayed during mid luteal phase (Figs. 1G, 3B). The immunostaining revealed by VEGFR-2 during follicular, early luteal and late luteal phases (Figs. 1I, J, L, 4B) was almost identical if expressed as values, while the staining in the mid luteal phase was weaker and significantly different from the other three.

Taken together, VEGF and both receptors displayed the strongest immunoreactivity in the follicular phase in endothelial cells, whereas in the smooth muscle cells only the pattern of VEGF immunostaining was similar. In all secretory phases, the immunoreactivity of VEGF and its receptors were different for endothelial and smooth muscle cells. The probable explanation is that they play different roles in the endothelium and muscular layer. Most biological functions of VEGF are mediated via VEGFR-2 and the role of VEGFR-1 is currently still to be discovered.

Discussion

This study presents the cellular localization of VEGF, VEGFR-1 and VEGFR-2 in the porcine broad ligament of the uterus. We have demonstrated the presence of these factors in the endothelial cells and muscular layer of the arteries of vascular subovarian plexus during the estrous cycle suggesting that their immunohistochemical response might be phase-related. Expression of VEGF, VEGFR-1 and VEGFR-2 in the reproductive vascular system still remains poorly understood, although it is proven that these factors play a crucial role in the female reproductive tract. Our study was focused on the uterine broad ligament vasculature supplying all reproductive organs with blood, oxygen, vasoactive molecules, hormones and other active substances. There is no data in the literature describing similar studies. Most of papers refer to reproductive organs: ovary and uterus of various animals and of humans [3, 23, 28, 30, 32, 42, 57] and these organs are particularly interesting in terms of VEGF localization and steroid hormones influencing this growth factor. Cooper and coworkers [9] described localization of VEGF and VEGFR-1 in human placenta and decidua. VEGF immunoreactivity was localized to placental macrophages and glandular epithelium and also maternal macrophages in decidua. The immunostaining of VEGFR-1 was observed in extravillous trophoblast. In human endometrium immunoreactivity of VEGF in glandular and stromal cells was significantly higher during the proliferative phase of the menstrual cycle [34]. The authors concluded that VEGF expression appeared to be under the influence of estrogens rather than progesterone. Similar results were demonstrated by Li *et al.* [27], who also immunolocalized VEGF in human endometrium. They detected no staining around the endometrial blood vessels throughout the secretory phase. The intense immunostaining was observed in stromal cells in the mid to late proliferative endometrium, which corresponds to the increase in the estradiol concentration. Observations by Winther and coworkers [57] confirmed the immunolocalization of VEGF and its both receptors in uterine luminal and glandular epithelium, trophoblast cells, endothelial cells and, for the first time, in smooth muscle cells of the vessel walls in pregnant and non-pregnant pigs. Endothelial cells of arteries displayed intense immunostaining for VEGFR-1 and VEGFR-2 during the late luteal phase of the estrous cycle. Evidence suggests that VEGF activity is spatially and temporally expressed during the cycle in ovary and uterus [39].

There are many studies supporting regulatory influence of estrogens on VEGF mRNA expression. Cullinan-Bove and Koos [10] have shown that VEGF is regulated by estradiol in the rat uterus *in vivo* inducing increase in uterine capillary permeability and growth. Ochoa *et al.* [37] investigated VEGF in the rat pituitary and concluded that VEGF in the cyclic uterus are under the control of estrogens. Reynolds *et al.* [42] documented rapid uterine response of VEGF to estradiol 17β in ovariectomized ewes.

Many investigations were also conducted *in vitro* on human uterine stromal cells [51], human carcinoma cell lines [6], and in breast cancer cells [48]. Isolated human endometrial cells treated with estradiol (E2) revealed significant, 3.1-fold increase in VEGF mRNA expression over the control value [51]. VEGF and its receptors as estrogen-responsive factors play an important role in endometrial angiogenesis, in the increase in microvascular permeability and hormone-dependent vascular growth. The study of Meduri *et al.* [32] was directed at determining the expression and modulation of VEGFR-1 and VEGFR-2 and at seeking their relation to the phase of the cycle. The immunohistochemical investigation revealed that the number of VEGFR-2-stained capillaries was maximal in the proliferative phase, whereas about 2-fold higher number of VEGFR-1 stained capillaries was observed in the secretory phase of the menstrual cycle.

Immunoreactivity of VEGFR-2 was higher in the midsecretory stage of the cycle, whereas VEGFR-1 staining was intense during the late proliferative

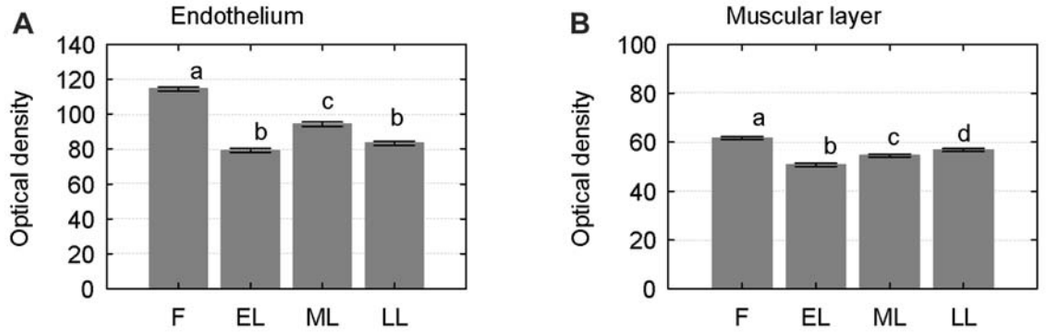

Fig. 2. Comparison of the intensity (optical density) of VEGF immunostaining in the endothelium (**A**) and muscular layer (**B**) of VSP arteries at various stages of the estrous cycle presented graphically. Different small letters indicate significant differences (P≤0.001). Each value is the mean ± SEM. Abbreviations for figures 2-4: F - follicular, EL - early luteal, ML - mid luteal, LL - late luteal phases of the estrous cycle.

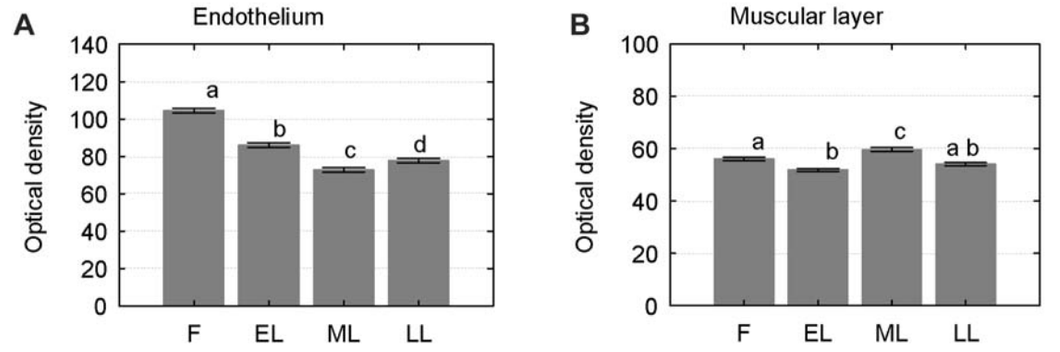

Fig. 3. Comparison of the intensity (optical density) of VEGFR-1 immunostaining in the endothelium (**A**) and muscular layer (**B**) of VSP arteries at various stages of the estrous cycle presented graphically. Different small letters indicate significant differences (P≤0.001). Each value is the mean ± SEM.

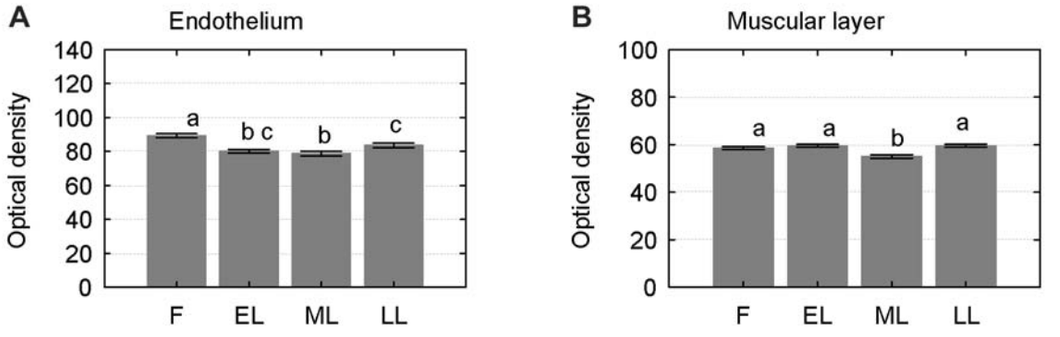

Fig. 4. Comparison of the intensity (optical density) of VEGFR-2 immunostaining in the endothelium (**A**) and muscular layer (**B**) of VSP arteries at various stages of the estrous cycle presented graphically. Different small letters indicate significant differences (P≤0.001). Each value is the mean ± SEM.

phase. Malamitsi-Puchner *et al.* [30] demonstrated that vascular expression of VEGFR-2 was high during the proliferative phase. These findings, as well as others suggest a relation of VEGF and its receptors immunoreactivity to the phases of the reproductive cycle.

A limited number of studies suggest that VEGF expression is increased not only by estrogens but also by progestins [7, 14, 17 , 25, 35], however, the effects of progestins on expression of VEGF and both receptors is not so clear as those of estrogens [19] because the knowledge of this mechanism is just beginning to

emerge. Greb *et al.* [14] observed intense VEGF immunostaining in the primate endometrial stroma after progestin treatment. According to Hyder and coworkers [17], progesterone increases VEGF protein expression in a human breast cancer cell line. Ancelin *et al.* [1] showed that progesterone selectively increased VEGF (189) expression in the human uterus.

The present study revealed that VEGF, VEGFR-1 and VEGFR-2 are expressed in the endothelial and smooth muscle cells in the area of VSP of cyclic pigs. We have observed a correlation between immunoreactivities of VEGF and its receptors in arterial walls and the estrous phases. Cyclic changes in expression of VEGF and both receptors may suggest that these factors remain under the influence of ovarian steroids. Positive expression of VEGF, VEGFR-1 and VEGFR-2 in the endothelial and smooth muscle cells gives evidence for the hypothesis that they also play a role to act in non-endothelial cells, stimulating different functions in the vasculature. Such results might also suggest that VEGF and both receptors remain under stronger influence of estrogens than progesterone. Still, all these results need further detailed investigation.

This study is the first step leading to further investigations that will demonstrate whether locally synthesized VEGF acting via its receptors is affected by ovarian steroid hormones and undergoes the cyclic changes in the uterine vasculature through the estrous cycle.

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References

[1] Ancelin M, Buteau-Lozano H, Meduri G, Osborne-Pellegrin M, Sordello S, Plouet J, Perrot-Applanat M (2002) A dynamic shift of VEGF isoforms with a transient and selective progesterone-induced expression of VEGF189 regulates angiogenesis and vascular permeability in human uterus. Proc Natl Acad Sci USA 99: 6023-6028

[2] Berse B, Brown LF, Van de Water L, Dvorak HF, Senger DR (1992) Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors. Mol Biol Cell 3: 211-220

[3] Bogic LV, Brace RA, Cheung CY (2001) Developmental expression of vascular endothelial growth factor (VEGF) receptors and VEGF binding in ovine placenta and fetal membranes. Placenta 22: 265-275

[4] Breier G, Clauss M, Risau W (1995) Coordinate expression of vascular endothelial growth factor receptor-1 (flt-1) and its ligand suggests a paracrine regulation of murine vascular development. Dev Dyn 204: 228-239

[5] Brock TA, Dvorak HF, Senger DR (1991) Tumor-secreted vascular permeability factor increases cytosolic Ca^{2+} and von Willebrand factor release in human endothelial cells. Am J Pathol 138: 213-221

[6] Charnock-Jones DS, Sharkey AM, Rajput-Williams J, Burch D, Schofield JP, Fountain SA, Boocock CA, Smith SK (1993) Identification and localization of alternately spliced mRNAs for vascular endothelial growth factor in human uterus and estrogen regulation in endometrial carcinoma cell lines. Biol Reprod 48: 1120-1128

[7] Classen-Linke I, Alfer J, Krusche CA, Chwalisz K, Rath W, Beier HM (2000) Progestins, progesterone receptor modulators, and progesterone antagonists change VEGF release of endometrial cells in culture. Steroids 65: 763-771

[8] Clauss M (1998) Functions of the VEGF receptor-1 (FLT-1) in the vasculature. Trends Cardiovasc Med 8: 241-245

[9] Cooper JC, Sharkey AM, McLaren J, Charnock-Jones DS, Smith SK (1995) Localization of vascular endothelial growth factor and its receptor, flt, in human placenta and decidua by immunohistochemistry. J Reprod Fertil 105: 205-213

[10] Cullinan-Bove K, Koos RD (1993) Vascular endothelial growth factor/vascular permeability factor expression in the rat uterus: rapid stimulation by estrogen correlates with estrogen-induced increases in uterine capillary permeability and growth. Endocrinology 133: 829-837

[11] Eriksson U, Alitalo K (1999) Structure, expression, and receptor-binding properties of novel vascular endothelial growth factors. Curr Top Microbiol Immunol 237: 41-57

[12] Ferrara N, Davis-Smyth T (1997) The biology of vascular endothelial growth factor. Endocr Rev 18: 4-25

[13] Gerber HP, Hillan KJ, Ryan AM, Kowalski J, Keller GA, Rangell L, Wright BD, Radtke F, Aguet M, Ferrara N (1999) VEGF is required for growth and survival in neonatal mice. Development 126: 1149-1159

[14] Greb RR, Heikinheimo O, Williams RF, Hodgen GD, Goodman AL (1997) Vascular endothelial growth factor in primate endometrium is regulated by oestrogen-receptor and progesterone-receptor ligands *in vivo*. Hum Reprod 12: 1280-1292

[15] Grosskreutz CL, Anand-Apte B, Duplaa C, Quinn TP, Terman BI, Zetter B, D'Amore PA (1999) Vascular endothelial growth factor-induced migration of vascular smooth muscle cells *in vitro*. Microvasc Res 58: 128-136

[16] Houck KA, Ferrara N, Winer J, Cachianes G, Li B, Leung DW (1991) The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. Mol Endocrinol 5: 1806-1814

[17] Hyder SM, Murthy L, Stancel GM (1998) Progestin regulation of vascular endothelial growth factor in human breast cancer cells. Cancer Res 58: 392-395

[18] Hyder SM, Nawaz Z, Chiapetta C, Stancel GM (2000) Identification of functional estrogen response elements in the gene coding for the potent angiogenic factor vascular endothelial growth factor. Cancer Res 60: 3183-3190

[19] Hyder SM, Stancel GM (1999) Regulation of angiogenic growth factors in the female reproductive tract by estrogens and progestins. Mol Endocrinol 13: 806-811

[20] Jakeman LB, Armanini M, Phillips HS, Ferrara N (1993) Developmental expression of binding sites and messenger ribonucleic acid for vascular endothelial growth factor suggests a role for this protein in vasculogenesis and angiogenesis. Endocrinology 133: 848-859

[21] Jingjing L, Xue Y, Agarwal N, Roque RS (1999) Human Muller cells express VEGF183, a novel spliced variant of vascular endothelial growth factor. Invest Ophthalmol Vis Sci 40: 752-759

[22] Joukov V, Pajusola K, Kaipainen A, Chilov D, Lahtinen I, Kukk E, Saksela O, Kalkkinen N, Alitalo K (1996) A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. EMBO J 15: 290-298

[23] Karuri AR, Kumar AM, Mukhopadhyay D (1998) Differential expression and selective localization of vascular permeability factor/vascular endothelial growth factor in the rat uterus during the estrous cycle. J Endocrinol 159: 489-499

[24] Kroll J, Waltenberger J (1999) A novel function of VEGF receptor-2 (KDR): rapid release of nitric oxide in response to

VEGF-A stimulation in endothelial cells. Biochem Biophys Res Commun 265: 636-639
[25] Lebovic DI, Shifren JL, Ryan IP, Mueller MD, Korn AP, Darney PD, Taylor RN (2000) Ovarian steroid and cytokine modulation of human endometrial angiogenesis. Hum Reprod 15, Suppl 3: 67-77
[26] Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N (1989) Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 246: 1306-1309
[27] Li XF, Gregory J, Ahmed A (1994) Immunolocalisation of vascular endothelial growth factor in human endometrium. Growth Factors 11: 277-282
[28] Licht P, Russu V, Lehmeyer S, Wissentheit T, Siebzehnrubl E, Wildt L (2003) Cycle dependency of intrauterine vascular endothelial growth factor levels is correlated with decidualization and corpus luteum function. Fertil Steril 80: 1228-1233
[29] Maglione D, Guerriero V, Viglietto G, Delli-Bovi P, Persico MG (1991) Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. Proc Natl Acad Sci USA 88: 9267-9271
[30] Malamitsi-Puchner A, Sarandakou A, Tziotis J, Stavreus-Evers A, Tzonou A, Landgren BM (2004) Circulating angiogenic factors during periovulation and the luteal phase of normal menstrual cycles. Fertil Steril 81: 1322-1327
[31] McMahon G (2000) VEGF receptor signaling in tumor angiogenesis. Oncologist 1: 3-10
[32] Meduri G, Bausero P, Perrot-Applanat M (2000) Expression of vascular endothelial growth factor receptors in the human endometrium: modulation during the menstrual cycle. Biol Reprod 62: 439-447
[33] Morbidelli L, Chang CH, Douglas JG, Granger HJ, Ledda F, Ziche M (1996) Nitric oxide mediates mitogenic effect of VEGF on coronary venular endothelium. Am J Physiol 270: H411-H415
[34] Naresh B, Sengupta J, Bhargava V, Kinra G, Hu J, Ghosh D (1999) Immunohistological localisation of vascular endothelial growth factor in human endometrium. Ind J Physiol Pharmacol 43: 165-170
[35] Nayak NR, Critchley HO, Slayden OD, Menrad A, Chwalisz K, Baird DT, Brenner RM (2000) Progesterone withdrawal upregulates vascular endothelial growth factor receptor type 2 in the superficial zone stroma of the human and macaque endometrium: potential relevance to menstruation. J Clin Endocrinol Metab 85: 3442-3452
[36] Obermair A, Obruca A, Pohl M, Kaider A, Vales A, Leodolter S, Wojta J, Feichtinger W (1999) Vascular endothelial growth factor and its receptors in male fertility. Fertil Steril 72: 269-275
[37] Ochoa AI, Mitchner NA, Paynter CD, Morris RE, Ben-Jonathan N (2000) Vascular endothelial growth factor in the rat pituitary: differential distribution and regulation by estrogen. J Endocrinol 165: 483-492
[38] Orlandini M, Marconcini L, Ferruzzi R, Oliviero S (1996) Identification of a c-fos-induced gene that is related to the platelet-derived growth factor/vascular endothelial growth factor family. Proc Natl Acad Sci USA 93: 11675-11680
[39] Phillips HS, Hains J, Leung DW, Ferrara N (1990) Vascular endothelial growth factor is expressed in rat corpus luteum. Endocrinology 127: 965-967
[40] Poltorak Z, Cohen T, Sivan R, Kandelis Y, Spira G, Vlodavsky I, Keshet E, Neufeld G (1997) VEGF 145, a secreted vascular endothelial growth factor isoform that binds to extracellular matrix. J Biol Chem 272: 7151-7158
[41] Reynolds LP, Kirsch JD, Kraft KC, Redmer DA (1998) Time-course of the uterine response to estradiol-17 in ovariectomized ewes: expression of angiogenic factors. Biol Reprod 59: 613-620
[42] Reynolds LP, Redmer DA (1998) Expression of the angiogenic factors, basic fibroblast growth factor and vascular endothelial growth factor, in the ovary. J Anim Sci 76: 1671-1681
[43] Roberts DM, Kearney JB, Johnson JH, Rosenberg MP, Kumar R, Bautch VL (2004) The vascular endothelial growth factor (VEGF) receptor Flt-1 (VEGFR-1) modulates Flk-1 (VEGFR-2) signaling during blood vessel formation. Am J Pathol 164: 1531-1535
[44] Roberts WG, Palade GE (1995) Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. J Cell Sci 108: 2369-2379
[45] Robinson C, Stringer S (2001) The splice variants of vascular endothelial growth factor (VEGF) and their receptors. J Cell Sci 114: 853-865.
[46] Rosen LS (2002) Clinical experience with angiogenesis signaling inhibitors: focus on vascular endothelial growth factor (VEGF) blockers. Cancer Contr 9: 36-43
[47] Rousseau S, Houle F, Huot J (2000) Integrating the VEGF signals leading to actin-based motility in vascular endothelial cells. Trends Cardiovasc Med 10: 321-327
[48] Ruohola JK, Valve EM, Karkkainen MJ, Joukov V, Alitalo K, Harkonen PL (1999) Vascular endothelial growth factors are differentially regulated by steroid hormones and antiestrogens in breast cancer cells. Mol Cell Endocrinol 149: 29-40
[49] Servos S, Zachary I, Martin JF (1999) VEGF modulates NO production: the basis of a cytoprotective effect? Cardiovasc Res 41: 509-510
[50] Sharkey AM, Day K, McPherson A, Malik S, Licence D, Smith SK, Charnock-Jones DS (2000) Vascular endothelial growth factor expression in human endometrium is regulated by hypoxia. J Clin Endocrinol Metab 85: 402-409
[51] Shifren JL, Tseng JF, Zaloudek CJ, Ryan IP, Meng YG, Ferrara N, Jaffe RB, Taylor RN (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
[52] Stavri GT, Hong Y, Zachary IC, Breier G, Baskerville PA, Yla-Herttuala S, Risau W, Martin JF, Erusalimsky JD (1995) Hypoxia and platelet-derived growth factor-BB synergistically upregulate the expression of vascular endothelial growth factor in vascular smooth muscle cells. FEBS Lett 358: 311-315
[53] Stein I, Neeman M, Shweiki D, Itin A, Keshet E (1995) Stabilization of vascular endothelial growth factor mRNA by hypoxia and hypoglycemia and coregulation with other ischemia-induced genes. Mol Cell Biol 15: 5363-5368
[54] Sugino N, Kashida S, Takiguchi S, Karube A, Kato H (2000) Expression of vascular endothelial growth factor and its receptors in the human corpus luteum during the menstrual cycle and in early pregnancy. J Clin Endocrinol Metab 85: 3919-3924
[55] Thomas KA (1996) Vascular endothelial growth factor, a potent and selective angiogenic agent. J Biol Chem 271: 603-606
[56] Waltenberger J, Claesson-Welsh L, Siegbahn A, Shibuya M, Heldin CH (1994) Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. J Biol Chem 269: 26988-26995
[57] Winther H, Ahmed A, Dantzer V (1999) Immunohistochemical localization of vascular endothelial growth factor (VEGF) and its two specific receptors, Flt-1 and KDR, in the porcine placenta and non-pregnant uterus. Placenta 20: 35-43
[58] Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J (2000) Vascular-specific growth factors and blood vessel formation. Nature 407: 242-248
[59] Zachary I, Gliki G (2001) Signaling transduction mechanisms mediating biological actions of the vascular endothelial growth factor family. Cardiovasc Res 49: 568-581

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