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The Clinical Significance of Serum Vascular Endothelial Growth Factor Levels Measured at Ovulation Triggering Day in Intrauterine Insemination Cycles

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자궁강 내 인공수정을 위한 과배란유도 시 hCG 투여 일에 측정한 혈중 Vascular Endothelial Growth Factor의 임상적 의의

김현준[1] · 지병철[2] · 서창석[2,3] · 김석현[3] · 최영민[3] · 김정구[3] · 문신용[3]

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목 적: 자궁강 내 인공수정을 위한 과배란유도 시 혈청 vascular endothelial growth factor (VEGF) 농도가 과배란유도의 결과를 반영할 수 있는지를 확인해 보고자 하였다.
연구방법: 과배란유도 후 자궁강 내 인공수정을 시행 받은 49명의 불임여성을 대상으로 hCG 투여 일에 혈청을 얻어 VEGF-A 및 estradiol 농도를 측정하였다. 과배란유도는 clomiphene citrate (100 mg/d on day 3~7)와 human menopausal gonadotropin (150 IU every other day starting on day 5) 병합요법을 이용하였다. hCG 투여 일에 17 mm 이상의 성숙난포 수와 자궁내막 두께를 동시에 측정하였다.
결 과: 혈청 VEGF-A 농도는 성숙난포 수, estradiol 농도 및 자궁내막 두께와는 무관하였던 반면 성숙난포 수와 estradiol 농도는 양의 비례관계를 보였다. 혈청 VEGF-A 농도는 성숙난포 수가 2개 이하인 저 반응군과 6개 이상인 고 반응 군에서 통계적으로 유의하지는 않지만 낮은 수치를 보였다.
결 론: 혈청 VEGF-A 농도는 자궁강 내 인공수정 시술 시 과배란유도의 결과와 무관한 것으로 사료되지만 저 반응 군과 고 반응 군에서 낮은 농도를 보이는 것으로 보아 이들을 대상으로 한 추가 연구가 필요할 것으로 판단된다.

중심단어: Vascular endothelial growth factor, 자궁강 내 인공수정, 과배란유도

Vascular endothelial growth factor (VEGF) is a 45 kD disulfide-linked homodimeric glycoprotein which is a powerful mediator for vascular permeability. VEGF is strongly implicated in the initiation and development of angiogenesis; it stimulates endothelial cell proliferation and increases capillary

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permeability.[1]

In normal menstruating women, VEGF is predominantly produced by granulosa and theca cells in response to follicle stimulating hormone (FSH), luteinizing hormone (LH), and human chorionic gonadotropin (hCG). It primarily stimulates the mitogenic properties of endothelial cells and provokes angiogenesis, transforming the poorly vascularized preovulatory follicle into the well-vascularized corpus luteum.[2,3]

Several authors mentioned that VEGF content within follicular fluids associated with progesterone secretion, embryo maturation, dose of administered gonadotropins and follicular hypoxia.[4,5]

There have been a few reports about correlation between serum or follicular fluid levels of VEGF and results of superovulation or pregnancy, and the results remains still controversial. Moreover, the study has been mainly restricted to IVF cycles. The present study was performed to investigate whether serum VEGF concentrations measured on the day of hCG administration reflect superovulation outcomes in IUI cycles.

MATERIALS AND METHODS

Forty-nine infertile couples with a duration of infertility of one year or more were recruited at the time of superovulation and IUI. All couples had undergone a proper infertility work-up and were determined to be candidates for IUI. The mean age of female was 32.2±2.7 years old; the mean duration of infertility was 3.9±2.0 years. We excluded couples when the female was > 37 years old, or had severe endometriosis (stage IV) or a basal serum FSH > 15 mIU/mL.

The infertility factors of the subjects were identified as unexplained (n=30), ovulatory (n=2), tubal (n=5), uterine (n=1), male factor (n=8) and endometriosis (n=3). The patients had no other diseases except infertility problem and had been not taken any medications.

Superovulation was performed using clomiphene citrate in combination with gonadotropin in an overlapping manner. Clomiphene citrate (Serophene®, Serono, Switzerland) 100 mg/d was given on day 3 to day 7 and human menopausal gonadotropin (hMG, Pergonal®, Serono, Switzerland) 150 IU was administered every other day starting on day 5 until hCG administration. When mature leading follicle(s) reached 19 mm in diameter and the urinary LH test was negative, urinary hCG (Profasi®, Serono, Switzerland) 5,000 IU was given, and then IUI was performed 36~40 hrs later. When the urinary LH test was positive, IUI was performed the next morning.

The luteal phase was supported by oral micronized (Utrogestan®, Laboratories Besins International, France) or intramuscular progesterone (Progest®, Samil Pharma, Korea). Clinical pregnancy was defined when an intrauterine gestational sac(s) was visible by ultrasonography.

The number of mature follicles (17 mm or more in diameter) and endometrial thickness were measured on the day of hCG administration. Blood samples were collected on the day of hCG administration and the serum aliquots were immediately separated, then frozen at -80℃ till assay. The concentrations of serum estradiol (TKE21, Diagnostic Products Corporation, USA) were measured using a radioimmunoassay (RIA) kit. VEGF-A165 concentrations were measured by commercial ELISA kit (Quantikine®, R&D systems, USA). The measurable range was 0~2,000 pg/mL. The coefficient of variation of intra-assay and inter-assay precision was 4.5~6.7% and 6.2~8.8%, respectively.

Data were analyzed with MedCalc Software (ver 6.10, Mariakerke, Belgium). The data were compared nonparametrically with the Kruskal-Wallis test for an overall comparison to predict significant differences between the groups. When possible significance was detected, the Wilcoxon test was

used between each group. Spearman correlation test was used to assess an association for different variables. Results were considered statistically significant when a P-value is <0.05.

RESULTS

Serum VEGF-A levels measured at hCG day did not correlate with the numbers of mature follicle count (Figure 1). They also did not correlate with peak serum estradiol levels (r=0.0709, p>0.05) nor endometrial thickness (r=0.551, p>0.05). However, serum estradiol level was positively associated with mature follicle count (r=0.4795, p<0.05) (Figure 2).

Since the study subjects per follicle count were rather small, we categorized the study subjects into three groups; group 1 included women with mature follicle count less than three (n=6), group 2 with three to five (n=26), and group 3 with more than five (n=17). There were no differences in female age, duration of infertility, dose of hMG and the

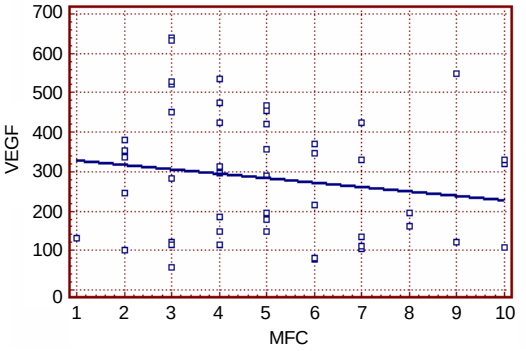

Figure 1. Relationship between serum levels of vascular endothelial growth factor (VEGF, pg/mL) and numbers of mature follicle count (MFC, 17 mm or more in diameter) measured at hCG day in forty-nine women undergoing superovulation and intrauterine insemination (r= -0.1600, p=0.2721).

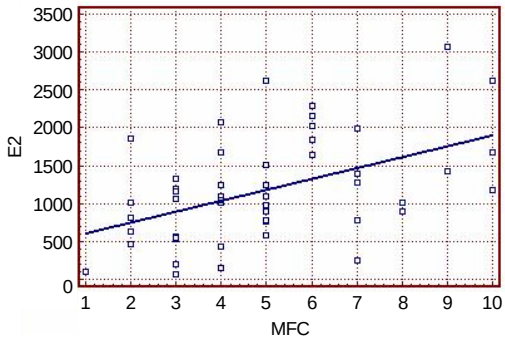

Figure 2. A strong positive relationship between serum estradiol (E_2, pg/mL) levels and number of mature follicle count (MFC, 17 mm or more in diameter) measured at hCG day in forty-nine women undergoing superovulation and intrauterine insemination (r=0.4795, p=0.0005).

Table 1. Clinical characteristics according to grouped follicle counts in forty-nine women undergoing superovulation and intrauterine insemination

	Group 1 (n=6)	Group 2 (n=26)	Group 3 (n=17)	P
Mature follicle count (≥17 mm)	1~2	3~5	6~10	
Female age (years)	33.7±3.4	32.5±2.6	31.2±2.4	0.1047
Duration of infertility (years)	4.5±2.8	3.2±1.1	4.6±2.6	0.2777
Dose of hMG (ampoule)	6.7±1.0	7.1±2.4	7.6±2.0	0.4986
hCG day	11.5±1.8	11.4±1.2	11.6±0.9	0.3772
Serum estradiol levels (pg/mL)	809.8±598.6[a]	994.0±575.8[b]	1617.1±709.9[c]	0.0344
Endometrial thickness (mm)	7.4±1.3	9.1±2.0	8.9±2.2	0.1126
Serum VEGF-A levels (pg/mL)	258.5±119.5	326.8±175.3	234.0±140.5	0.1901

Values are mean ± SD.
P-values are calculated by Kruskal-Wallis test.
hMG = human menopausal gonadotropin; hCG = human chorionic gonadotropin;
VEGF-A = vascular endothelial growth factor-A
P-values by Wilcoxon test: [a-c] = 0.0209, [b-c] = 0.0040

day of hCG administration among the three groups (Table 1). Serum estradiol levels were significantly higher in group 3, but endometrial thickness was not different between groups. Serum VEGF-A levels were not different among three groups, but there was a tendency of lower serum VEGF-A levels in women with mature follicle count less than three or women with more than five.

DISCUSSION

In the present study, serum VEGF-A levels measured on hCG triggering day did not have an association with superovulation outcome in women undergoing IUI cycles. This suggests that serum VEGF level is not a proper marker reflecting ovarian response and there is no clinical role in women undergoing superovulation.

However, there was a tendency of lower serum VEGF-A levels in women with mature follicle count less than three or women with more than five. This finding suggests that those women showing extreme response to superovulation may relate with abnormal angiogenesis. Therefore, the exact role of serum VEGF should be further verified in larger populations, especially in poor responders or high responders.

Although the majority of studies focused on the clinical significance of serum VEGF concentrations in IVF cycles, our study was performed in IUI cycles combined with superovulation. Previous investigator suggested that a close association between increased VEGF expression during the follicular phase and the number of follicles destined for ovulation and the VEGF levels may be a predictor of superovulation outcome.[6~9] However, some authors suggested that elevated follicular fluid VEGF levels was associated with diminished pregnancy potential, hypothesizing a relative follicular hypoxia because of a hypoxic follicular environment.[10,11] Therefore, the role of VEGF in superovulation is still controversial.

Superovulation for assisted reproduction is currently a common therapeutic procedure in many infertility clinics. Follicular development after superovulation is a complex process regulated by multiple local factors under the various influence of cyclic hormone. The ovary is highly vascular and has high rates of blood flow since it is supplied directly by aorta. Within the ovary, the vascular complex is formed on a cyclic basis. Therefore, angiogenesis is an important component of the growth and function of this reproductive organ.

The smaller primordial follicles are dependent on their proximity to the stromal vessels because of absence of an independent capillary network. The primary follicles develop an initial vascular supply consisting of a few arterioles terminating in an increasingly complex network as the follicle continues to develop. Hence active blood supply is essential for the induction of good quality oocytes via an appropriate follicular development.[12,13]

VEGF is a potent and specific stimulator of angiogenesis as a peptide growth factor with at least five isoforms. The VEGF, a family of dimeric glycoproteins denoted as VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor, have potent angiogenic, mitogenic, and vascular permeability activities. The VEGF-A is expressed in humans as five splice variants of a single gene, with 121, 145, 165, 189, and 206 amino acid residues.[14,15] The VEGF proteins bind with different ligand specify through high-affinity receptors designated VEGFR-1 (Flt 1), VEGFR-2 (Flk-1/KDR), VEGFR-3 (Flt-4) and neutropilin-1.[16]

Follicular angiogenesis may be a determinant of follicular development during the periovulatory phase, and VEGF may play important roles in regulating follicular angiogenesis. Permeabilizing and survival actions of VEGF are fundamental for appropriate development and functioning of follicle and corpus luteum during the menstrual period or

pregnant period.[18,19] The degree of vascular development is follicle specific and differences among follicles might reflect their unique abilities to regulate angiogenic growth factors production by the follicle cells in response to hypoxia.[20]

Increased levels of VEGF in follicular fluid after controlled ovarian stimulation for IVF has been reported to be associated with fewer retrieved oocytes, fewer mature oocytes and fewer embryos.[21] Decreased follicular fluid and serum VEGF and elevated follicular fluid inhibin A and B are associated with better ovarian response and high pregnancy rate.[11,21] Dorn et al. also reported that a direct association between serum and follicular fluid VEGF levels.[22]

Gene knockout studies have provided interesting evidence for a central role of VEGF in angiogenesis. Homozygous gene knockouts for VEGF were lethal by about day 11 of gestation, and these embryos showed significant cardiovascular defects, such as abnormal development of the heart, aorta, major vessels and placenta. Heterozygous VEGF gene knockout embryos, which expressed VEGF but at reduced levels, exhibited similar defects in fetal and placental angiogenesis, and also died by about day 11 of pregnancy. It was suggested that threshold levels of VEGF must be achieved for normal vascular development to occur.[23,24]

In conclusion, serum VEGF-A levels measured at ovulation trigger did not correlate with superovulation outcome in IUI women such as the numbers of mature follicle count and serum estradiol levels. However, there was a tendency of lower VEGF-A level in poor and high responder; this finding suggests that those with extreme response to superovulation may relate with abnormal angiogenesis. Therefore, the exact role of serum VEGF should be further verified in larger populations.

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= Abstract =

Objective: The objective of this study was to investigate whether serum levels of vascular endothelial growth factor (VEGF) measured at ovulation triggering day reflect ovarian response in intrauterine insemination (IUI) cycles.

Methods: Forty-nine infertile women who undergoing superovulation and IUI were included. Superovulation was performed using clomiphene citrate (100 mg/d on day 3~7) in combination with human menopausal gonadotropin (150 IU every other day starting on day 5). Serum samples were obtained on the day of hCG administration and the levels of VEGF-A and estradiol were measured. The numbers of mature follicle ≥17 mm in diameter were also counted.

Results: Serum VEGF-A levels did not correlate with the numbers of mature follicle count nor serum estradiol levels. Serum estradiol level was positively associated with mature follicle count. Serum VEGF-A levels tended to be lower in women with mature follicle count less than three or women with more than five.

Conclusion: Our results indicate that serum VEGF-A levels do not have an association with superovulation outcome in IUI cycles. However, a tendency of lower VEGF-A level in poor and high responder suggests that those with extreme response to superovulation may be related with abnormal angiogenesis. Further studies should be warranted in larger populations.

Key Words: Vascular endothelial growth factor, Intrauterine insemination, Superovulation