

# Endometrial growth and uterine blood flow: a pilot study for improving endometrial thickness in the patients with a thin endometrium

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**Objective:** To examine whether thin endometria can be improved by increasing uterine radial artery (uRA) blood flow.

**Design:** A prospective observational study.

**Setting:** University hospital and city general hospital.

**Patient(s):** Sixty-one patients with a thin endometrium (endometrial thickness [EM] <8 mm) and high radial artery-resistance index of uRA (RA-RI  $\geq$  0.81).

**Intervention(s):** Vitamin E (600 mg/day, n = 25), L-arginine (6 g/day, n = 9), or sildenafil citrate (100 mg/day, intravaginally, n = 12) was given.

**Main Outcome Measure(s):** EM and RA-RI were assessed by transvaginal color-pulsed Doppler ultrasound.

**Result(s):** Vitamin E improved RA-RI in 18 (72%) out of 25 patients and EM in 13 (52%) out of 25 patients. L-arginine improved RA-RI in eight (89%) out of nine patients and EM in six (67%) patients. Sildenafil citrate improved RA-RI and EM in 11 (92%) out of 12 patients. In the control group (n = 10), who received no medication to increase uRA-blood flow, only one (10%) patient improved in RA-RI and EM. The effect of vitamin E was histologically examined in the endometrium (n = 5). Vitamin E improved the glandular epithelial growth, development of blood vessels, and vascular endothelial growth factor protein expression in the endometrium.

**Conclusion(s):** Vitamin E, L-arginine, or sildenafil citrate treatment improves RA-RI and EM and may be useful for the patients with a thin endometrium. (Fertil Steril® 2010;93:1851–8. ©2010 by American Society for Reproductive Medicine.)

**Key Words:** Thin endometrium, uterine blood flow, radial artery, vitamin E, sildenafil citrate

Adequate growth of the endometrium is indispensable for successful pregnancy. We often see patients with a thin endometrium, which may be caused by impairment of the normal process of endometrial growth. Low pregnancy rates are noted in patients with a thin endometrium (1–5). However, improving endometrial growth in patients with a thin endometrium is very difficult. In addition, little information is available regarding the factors responsible for impaired endometrial growth in patients with a thin endometrium.

Very recently, we showed the pathophysiological features of thin endometrium and suggested that a thin endometrium may be due to high blood flow impedance of uterine radial

arteries, which are in the lower extremity of uterine arteries (5). Uterine blood flow is an important factor for endometrial growth (6, 7). Interestingly, we found that blood flow impedance of uterine radial arteries remains high throughout the menstrual cycle in patients with a thin endometrium, suggesting that high blood flow impedance of uterine radial arteries is associated with poor endometrial growth in patients with a thin endometrium (5). In our recent report (5), we raised our hypothesis for the pathophysiology of thin endometrium as follows: high blood flow impedance of uterine radial arteries, which could be a trigger, impairs the growth of the glandular epithelium and results in a decrease in vascular endothelial growth factor (VEGF) expression, which is a key factor for regulating angiogenesis in the human endometrium (8–10). Low VEGF levels cause poor vascular development, which in turn decreases blood flow in the endometrium. The vicious circle leads to a thin endometrium. Thus, it is likely that high blood flow impedance of uterine radial arteries is involved in the etiology of a thin endometrium. We therefore decided to study whether thin endometria can be improved by increasing uterine radial artery blood flow.

For this purpose, we focused on vitamin E and potential nitric oxide (NO) donors such as L-arginine and sildenafil

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citrate to increase uterine radial artery blood flow. Vitamin E has been shown to improve capillary blood flow in a variety of organs not only by inhibiting the breakdown of lipids in red blood cell membranes (11, 12) but also by protecting the endothelium from oxidative stress (13, 14). NO release from vascular endothelial cells leads to the relaxation of vascular smooth muscle mainly by activating cyclic guanosine monophosphate (cGMP) (15). Sildenafil citrate, a type 5 specific phosphodiesterase inhibitor, prevents the breakdown of cGMP and potentiates the effect of NO on vascular smooth muscle (16). L-arginine, a substrate of NO, increases hepatic and limb blood flow (17, 18). The present study was undertaken as a pilot study to investigate whether vitamin E, L-arginine, or sildenafil citrate treatment has a potential to increase the blood flow of uterine radial arteries and to improve endometrial growth in patients with a thin endometrium.

## MATERIALS AND METHODS

The project was reviewed and approved by the Institutional Review Board of Yamaguchi University Graduate School of Medicine. Informed consent was obtained from the patients before collection of any tissue samples for this study.

### Patients

A total of 61 women with a history of infertility, who had both a thin endometrium (endometrial thickness <8 mm) and high blood flow impedance of uterine radial arteries (radial artery-resistance index [RA-RI]  $\geq 0.81$ ), were recruited into this study. Endometrial thickness and uterine RA-RI were measured in the late follicular phase (1–2 days before ovulation),

and their cutoff values were determined as described below. The patients had normal menstrual cycles (26–35 days) and were 23–44 years old. They were nonsmokers and free from major medical illnesses including hypertension. Women were excluded if they had myoma, adenomyosis, or congenital uterine anomaly or had chronic use of any medication including nonsteroidal anti-inflammatory agents.

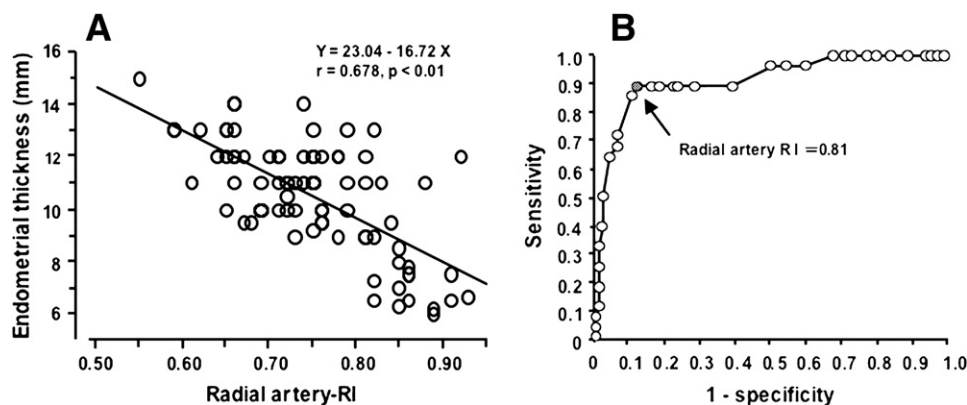
After a longitudinal view of the uterus was obtained, the thickness of the endometrium was measured at the maximal distance between each myometrial/endometrial interface using vaginal ultrasound. The cutoff value of endometrial thickness between normal and thin endometrium was defined as 8 mm based on our IVF-ET data, as reported elsewhere (5).

Blood flow impedance of uterine radial arteries was evaluated with the use of a computerized vaginal ultrasound with an integrated pulsed Doppler vaginal scanner (Aloka, Tokyo, Japan) and assessed as RI, as reported elsewhere (5, 19). The RA blood flow pattern was determined by demonstrating pulsatile color signals in the myometrium. After confirming that waveforms were continuous, an average of three to five cardiac cycles was selected for calculation of the RI. The mean of the two points of RA-RI was used for statistical analyses. Since the interobserver coefficient of variation for Doppler flow measurements in the present study was less than 10%, which is consistent with the report by Ziegler et al. (20), the Doppler flow measurements were judged to be reproducible.

There was a significant negative correlation between endometrial thickness and RA-RI, which were measured in the late follicular phase (1–2 days before ovulation; Fig. 1A). Receiver operating characteristic curve (ROC) analysis was

## FIGURE 1

Correlation between blood flow impedance of uterine radial arteries and endometrial thickness. (A) Correlation between uterine RA-RI and endometrial thickness ( $n = 66$ ). (B) ROC curve analysis. RA-RI and endometrial thickness were measured for infertility patients in the late follicular phase (1–2 days before ovulation). Endometrial thickness was significantly and negatively correlated with RA-RI (single regression analysis). ROC curve analysis was performed to determine the cutoff value of the RA-RI providing the best values of sensitivity and specificity for determination of normal and thin endometrium. The cutoff value of 0.81 provided the best combination, with 89.3% sensitivity and 87.6% specificity to discriminate between normal and thin endometrium.



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performed to determine the cutoff value of the RA-RI providing the best value of the sensitivity and the specificity for determination of normal and thin endometrium. The cutoff value of 0.81 provided the best combination, with 89.3% sensitivity and 87.6% specificity to discriminate between normal and thin endometrium (Fig. 1B).

### Vitamin E Treatment

Twenty-five patients who showed a thin endometrium (<8 mm) and high RA-RI ( $\geq 0.81$ ) in the late follicular phase (1–2 days before ovulation) were given vitamin E (600 mg/day, 3 times/day orally; Eisai Co., Ltd., Tokyo, Japan) from the first day of the subsequent menstrual cycle throughout the menstrual cycle because we found that blood flow impedance of radial arteries remained high from the start of the menstrual cycle in patients with a thin endometrium (5). The concomitant treatment for infertility during vitamin E treatment includes natural cycles ( $n = 16$ ), clomiphene treatment cycles ( $n = 3$ ), and hMG-hCG treatment cycles ( $n = 6$ ). First, to evaluate the effect of vitamin E, endometrial thickness and RA-RI were measured in the late follicular phase (1–2 days before ovulation) or at the day of hCG injection for ovulation induction when follicles reached 18 mm or more in diameter and were compared with those of the previous cycle without vitamin E treatment. The numbers of follicles (15 mm or greater) or serum  $E_2$  concentrations were also measured 1–2 days before ovulation or at the day of hCG injection for ovulation induction and were compared between the previous cycle and the subsequent treatment cycle with vitamin E in each treatment group for infertility. As controls, 10 patients with a thin endometrium (<8 mm) and high RA-RI ( $\geq 0.81$ ) in the late follicular phase received no medication to increase RA blood flow during the subsequent menstrual cycle. The treatment for infertility in the control group includes natural cycles ( $n = 3$ ), hMG-hCG treatment cycles ( $n = 4$ ), and IVF-ET treatment cycles ( $n = 3$ ).

In addition, to examine the effect of vitamin E on the histological parameters such as the growth of glandular epithelium, the number of blood vessels, and VEGF expression, endometrial biopsy specimens were obtained in the midluteal phase (days 6–8 after ovulation) before and during vitamin E treatment in five patients with a thin endometrium. The patients had no concomitant treatments for infertility. The day of ovulation was determined by ultrasound, measurement of urinary LH, and basal body temperature records. Tissue samples were washed with saline to remove blood, immediately frozen in liquid nitrogen, and stored at  $-80^\circ\text{C}$ . To evaluate the growth of the glandular epithelium, the area of glandular epithelial cells was measured within a unit area ( $0.318 \text{ mm}^2/\text{field}$ ) using ImageJ (Wayne Rasband, National Institutes of Health, Bethesda, MD) and expressed as the percentage of the area of glandular cells per unit area as reported elsewhere (5). The number of blood vessels was quantified with immunohistochemistry for CD34 according to the method that we reported elsewhere (5, 21). VEGF expression was evaluated by Western blot analysis as reported elsewhere (5, 9).

### L-Arginine Treatment

Nine patients who showed a thin endometrium (<8 mm) and high RA-RI ( $\geq 0.81$ ) in the late follicular phase were given l-arginine (6 g/day, 4 times/day orally; Now Foods, Bloomingdale, IL) from the first day of the subsequent menstrual cycle until the day of hCG injection for ovulation induction. Endometrial thickness and RA-RI were measured at the day of hCG injection and were compared with those of the previous cycle without l-arginine treatment. The concomitant treatment for infertility includes natural cycles ( $n = 7$ ) and hMG-hCG treatment cycles ( $n = 2$ ). The numbers of follicles (15 mm or greater) and serum  $E_2$  concentrations were also measured at the day of hCG injection and were compared between the previous cycle and the subsequent treatment cycle with l-arginine in each treatment group for infertility.

### Sildenafil Citrate (Viagra) Treatment

Twelve patients with a thin endometrium (<8 mm) and high RA-RI ( $\geq 0.81$ ) in the late follicular phase were given sildenafil citrate (Viagra, 100 mg/day, 4 times per day, intravaginally [self-administered]; Pfizer Inc., New York, NY) from the first day of the subsequent menstrual cycle until the day of hCG injection in the IVF-ET treatment cycle. Ovarian stimulation was performed with the long protocol with a GnRH agonist as reported elsewhere (22). The sildenafil suppositories were prepared from the oral tablets. The tablets were mashed and dissolved in Hosco S-55 (25 mg sildenafil/1.35 g Hosco S-55; MarUishi Co., Osaka, Japan). Endometrial thickness and RA-RI were measured at the day of hCG injection and were compared with those of the previous cycle without sildenafil citrate treatment. The numbers of follicles (15 mm or greater) and serum  $E_2$  concentrations at the day of hCG injection were also compared between the previous cycle and the subsequent treatment cycle with sildenafil citrate.

### Statistical Analysis

Statistical analysis was carried out using the software program SPSS for Windows, version 13.0 (SPSS, Chicago). The Mann-Whitney *U*-test, single regression analysis, and  $\chi^2$ -test were used as appropriate.  $P < .05$  was considered statistically significant.

## RESULTS

### Vitamin E Treatment

Twenty-five patients who had a thin endometrium (<8 mm) and high RA-RI ( $\geq 0.81$ ) in the late follicular phase of the previous cycle were given vitamin E during the subsequent treatment cycle. Eighteen (72%) out of 25 patients showed improved RA-RI (<0.81; Table 1). Thirteen (52%) patients developed an endometrium of more than 8 mm, and five (20%) patients conceived during the vitamin E treatment cycle (Tables 1 and 2). Each of these effects of vitamin E was statistically significant between the treatment cycle and the previous cycle (Table 1). In the control group, only one (10%) patient out of 10 improved in RA-RI and endometrial

**TABLE 1****Effects of vitamin E, l-arginine, or sildenafil citrate on RA resistance index and endometrial thickness in the patients with a thin endometrium.**

	n	RA-RI			EM (mm)			
		Previous cycle	Treatment cycle	No. of < 0.81 (%)	Previous cycle	Treatment cycle	No. of > 8 mm (%)	No. of pregnancies (%)
Control	10	0.866 (0.814–0.908)	0.866 (0.729–0.895)	1 (10)	7.3 (6.0–7.8)	6.8 (6.0–10.0)	1 (10)	0 (0)
Vitamin E	25	0.861 (0.812–0.948)	0.780 <sup>a</sup> (0.690–0.895)	18 <sup>c</sup> (72)	7.2 (5.5–7.8)	8.3 <sup>b</sup> (5.2–11.0)	13 <sup>d</sup> (52)	5 (20)
L-arginine	9	0.842 (0.812–0.879)	0.713 <sup>a</sup> (0.649–0.900)	8 <sup>c</sup> (89)	7.4 (5.5–7.8)	8.0 <sup>b</sup> (6.0–9.2)	6 <sup>d</sup> (67)	1 (11)
Sildenafil citrate	12	0.872 (0.815–0.931)	0.714 <sup>a</sup> (0.603–0.815)	11 <sup>c</sup> (92)	7.1 (5.5–7.8)	9.4 <sup>a</sup> (7.0–12.0)	11 <sup>c</sup> (92)	6 (50)

Note: Fifty-six patients with a thin endometrium (endometrial thickness [EM] <8 mm) and high RA-RI ( $\geq 0.81$ ) were recruited in this study. EM and RA-RI were assessed by transvaginal color-pulsed Doppler ultrasound. To study whether thin endometrium is improved by increasing RA blood flow, vitamin E (600 mg/day, n = 25) was given throughout the menstrual cycle, and l-arginine (6 g/day, n = 9) or sildenafil citrate (Viagra, 100 mg/day, intravaginally, n = 12) was given until ovulation induction. Controls received no medication to increase uterine blood flow (n = 10). EM and RA-RI were compared between the treatment cycle and the previous cycle. One (10%) patient out of 10 spontaneously improved in RA-RI and EM (control group). By vitamin E treatment, 18 (72%) out of 25 patients showed improved RA-RI, 13 (52%) patients developed an endometrium of more than 8 mm, and five patients conceived during the treatment cycle. L-arginine treatment improved RA-RI in eight (89%) out of nine patients and EM in six (67%) out of nine patients, and one patient conceived. Sildenafil citrate treatment improved RA-RI and EM in 11 (92%) patients out of 12, and six patients conceived during the treatment cycle. Values show median with ranges.

<sup>a</sup>  $P < .01$ .

<sup>b</sup>  $P < .05$  versus previous cycle (Mann-Whitney  $U$ -test).

<sup>c</sup>  $P < .01$ .

<sup>d</sup>  $P < .05$  versus control ( $\chi^2$ -test).

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**TABLE 2****Effects of the treatment with vitamin E, l-arginine, or sildenafil citrate on follicular growth or serum E<sub>2</sub> levels.**

Treatments to increase blood flow	Fertility treatments	No. of preovulatory follicles 15 mm or greater		Serum E <sub>2</sub> , pg/mL		No. of pregnancies
		Previous cycle	Treatment cycle	Previous cycle	Treatment cycle	
Vitamin E	Natural cycle (n = 16)	1.0 (1–1)	1.0 (1–1)	NM	NM	2
	Clomiphene (n = 3)	2.3 (2–3)	2.3 (1–3)	NM	NM	2
	hMG-hCG (n = 6)	4.8 (3–10)	4.3 (2–8)	1138 (680–2015)	887 (290–1600)	1
L-arginine	Natural cycle (n = 7)	1.0 (1–1)	1.0 (1–1)	NM	NM	0
	hMG-hCG (n = 2)	3.0 (3–3)	3.0 (3–3)	593 (505–680)	575 (495–655)	1
Sildenafil citrate	IVF-ET (n = 12)	4.0 (1–10)	3.9 (2–8)	1289 (346–2609)	1153 (190–2579)	6

Note: Vitamin E (600 mg/day, n = 25) was given throughout the menstrual cycle, and l-arginine (6 g/day, n = 9) or sildenafil citrate (Viagra, 100 mg/day, intravaginally, n = 12) was given until the ovulation induction for the patients with a thin endometrium. The concomitant treatment for infertility during vitamin E treatment includes natural cycles (n = 16), clomiphene cycles (n = 3), and hMG-hCG cycles (n = 6). The concomitant treatment for infertility during l-arginine treatment includes natural cycles (n = 7) and hMG-hCG cycles (n = 2). Sildenafil citrate was used for IVF-ET. The numbers of follicles (15 mm or greater) or serum E<sub>2</sub> concentrations were measured 1–2 days before ovulation or at the day of hCG injection for ovulation induction and were compared between the previous cycle and the subsequent treatment cycle with vitamin E, l-arginine, or sildenafil citrate in each treatment group for infertility. The numbers of pregnancy were also shown in each treatment for infertility. Values show medians or means with ranges. NM = not measured.

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thickness, suggesting that vitamin E significantly improved RA-RI and endometrial thickness compared with the controls (Table 1).

Five patients with a thin endometrium and high RA-RI provided biopsy tissue samples in the midluteal phase before and during vitamin E treatment. All five patients showed improvement of RA-RI (<0.81) and endometrial thickness (>8 mm; Table 3). Vitamin E improved the growth of the glandular epithelium and the development of blood vessels (Table 3). VEGF protein expression was also increased by vitamin E treatment (Fig. 2). Each of these effects of vitamin E was statistically significant between the treatment cycle and the previous cycle (Table 3 and Fig. 2).

### L-Arginine Treatment

L-arginine treatment improved RA-RI (<0.81) in eight (89%) out of nine patients. Six (67%) patients developed an endometrium of more than 8 mm, and one (11%) patient conceived during the l-arginine treatment cycle (Tables 1 and 2). Each of these effects of l-arginine was statistically significant between the treatment cycle and the previous cycle (Table 1).

There was also a significant difference in improvement of RA-RI (<0.81) and endometrial thickness (>8 mm) between the l-arginine group and the control group (Table 1).

### Sildenafil Citrate Treatment

Twelve patients with a thin endometrium and high RA-RI were given sildenafil suppositories in the IVF-ET treatment cycle. Eleven (92%) patients showed improved RA-RI (<0.81) and endometrial thickness (>8 mm) after sildenafil citrate treatment (Table 1). Six (50%) patients conceived during the sildenafil treatment cycle (Tables 1 and 2). Each of these effects of sildenafil citrate was statistically significant between the treatment cycle and the previous cycle (Table 1). In the control group, three patients out of 10 received IVF-ET, and four patients received hMG-hCG therapy, but they showed no improvement in RA-RI and endometrial thickness.

### Effects of the Treatment with Vitamin E, l-Arginine, or Sildenafil Citrate on Follicular Growth or Serum E<sub>2</sub> Levels

Treatments with vitamin E, l-arginine, or sildenafil citrate caused no significant effects on the numbers of preovulatory



**TABLE 3****Effects of vitamin E treatment on RA resistance index, endometrial growth, and vascular development in the patients with a thin endometrium.**

	Previous cycle	Vitamin E treatment cycle
RA-RI	0.861 (0.831–0.955)	0.742 <sup>a</sup> (0.677–0.769)
Endometrial thickness, mm	6.6 (5.9–7.6)	9.1 <sup>a</sup> (8.0–10.0)
Area of glandular epithelial cells per unit area, %	16.3 (11.0–18.4)	34.1 <sup>a</sup> (24.5–36.1)
No. of blood vessels/mm <sup>2</sup>	120.3 (49.3–180.1)	200.0 <sup>a</sup> (150.3–222.0)

*Note:* Five patients with a thin endometrium (EM <8 mm) and high RA-RI ( $\geq 0.81$ ) were recruited in this study. To examine the effect of vitamin E on the histological parameters such as the growth of glandular epithelium and the number of blood vessels, vitamin E (600 mg/day) was given throughout the menstrual cycle, and endometrial biopsy specimens were obtained in the midluteal phase before and during vitamin E treatment. To evaluate the growth of the glandular epithelium, the area of glandular epithelial cells was measured within a unit area (0.318 mm<sup>2</sup>/field) and expressed as the percentage of the area of glandular cells per unit area. The number of blood vessels was quantified using immunohistochemistry for CD34. Each parameter was compared between the treatment cycle and the previous cycle. Values show medians with ranges. Data were analyzed by the Mann-Whitney *U*-test.

<sup>a</sup> *P* < .05 versus previous cycle.

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follicles or serum E<sub>2</sub> concentrations in the natural cycles, clomiphene cycles, hMG-hCG cycles, and IVF-ET cycles (Table 2).

## DISCUSSION

The present study showed that treatments with vitamin E, l-arginine, or sildenafil citrate significantly improved uterine RA-RI and endometrial thickness in the patients with a thin endometrium. Uterine blood flow is an important factor for endometrial growth (6, 7). The present result also showed that uterine RA-RI is negatively correlated with endometrial thickness. It is therefore likely that vitamin E, l-arginine, or sildenafil citrate improves endometrial growth by increasing uterine RA blood flow. Very recently, we reported the pathophysiological features of thin endometrium and suggested that a thin endometrium may be due to high blood flow impedance of uterine radial arteries (5). The present result that increasing uterine RA blood flow improved endometrial growth strongly suggests that high blood flow impedance of uterine radial arteries is involved in the pathogenesis of poor endometrial growth in patients with a thin endometrium.

In our recent report (5), we raised our hypothesis for pathophysiology of thin endometrium as follows: high blood flow impedance of uterine radial arteries, which could be a trigger, impairs the growth of the glandular epithelium and results in a decrease in VEGF expression. Low VEGF levels cause poor vascular development, which in turn decreases blood flow in the endometrium. The vicious circle leads to a thin endometrium. In this study, we examined whether impaired growth of glandular epithelium, poor angiogenesis, and low VEGF expression in the endometrium of the patient with a thin endometrium can be improved by increasing uterine RA blood flow. The histological analysis of the present study clearly

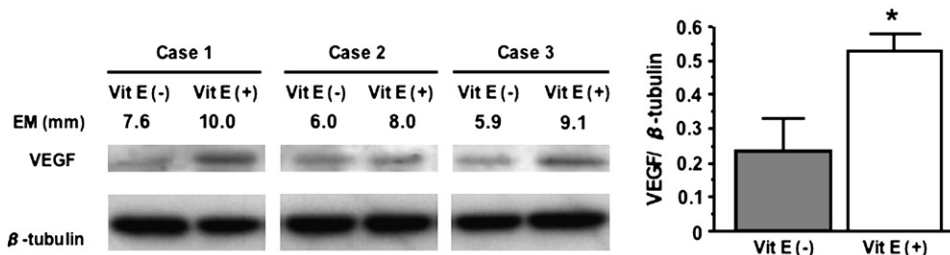
showed that vitamin E treatment improved the growth of glandular epithelium, angiogenesis, and VEGF expression in the endometrium as well as the RA-RI, suggesting that the pathophysiology of thin endometrium may be due to the decreased uterine RA blood flow as we hypothesized.

Although the literature on the treatment for thin endometrium is rather sparse, our results are partially consistent with some previous reports. Treatments by combination of pentoxifylline and vitamin E improved the pregnancy rate in patients with a thin endometrium by increasing the endometrial thickness (23). Vaginal administration of sildenafil citrate improved endometrial growth and pregnancy rates in patients with a thin endometrium (24–26), although there is a conflicting report (27). Unfortunately, these studies did not focus on the relationship between endometrial growth and uterine blood flow. Aspirin has also been shown to reduce uterine vascular resistance (28). Low-dose aspirin increased the pregnancy rate of patients in an intrauterine insemination program (29) or in an oocyte donation program (30), however, significant improvement in endometrial thickness and uterine blood flow by low-dose aspirin treatment was not found (29, 30). Check et al. (31) reported no positive effects of low-dose aspirin therapy on pregnancy rates after frozen ET. Thus, the present study is, to our knowledge, the first report to suggest a close relationship among endometrial growth, uterine blood flow, and treatments that improve uterine blood flow.

Since the patients in each group of this study received different fertility treatments, we cannot neglect the influence of the heterogeneity of the study group. In addition, there seems to be a possibility that vitamin E, l-arginine, or sildenafil citrate has the potential to increase ovarian blood flow and works through stimulating follicular growth and increasing serum E<sub>2</sub> levels. In fact, we recently found that corpus luteum

## FIGURE 2

Effects of vitamin E treatment on VEGF expression in the endometrium of the patients with a thin endometrium. Vitamin E (600 mg/day) was given to five patients with a thin endometrium (endometrial thickness  $<8$  mm) and high RA-RI ( $\geq 0.81$ ) throughout the menstrual cycle, and endometrial biopsy specimens were obtained in the midluteal phase before and during vitamin E treatment. VEGF expression was analyzed by Western blotting. Representative immunoblots of VEGF protein expression before [vit E (-)] and during [vit E (+)] vitamin E treatment are shown from three patients. The right panel shows the quantification of immunoblot signals (ratio of VEGF to  $\beta$ -tubulin), mean  $\pm$  SEM. \* $P < .05$  vs. vit E (-) (Mann-Whitney *U*-test).



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blood flow is closely associated with luteal function (32) and that vitamin E or L-arginine treatment improves corpus luteum blood flow in our unpublished data. However, in the present study, vitamin E, L-arginine, or sildenafil citrate caused no significant effects on the numbers of preovulatory follicles or serum  $E_2$  concentrations in the natural cycles, clomiphene cycles, hMG-hCG cycles, and IVF-ET cycles. The finding suggests that vitamin E, L-arginine, or sildenafil improved endometrial growth through improving endometrial blood flow rather than stimulating follicular growth or increasing serum  $E_2$  levels. In addition, this study shows that endometrial growth hardly improves spontaneously regardless of the method of fertility treatments, including the hMG-hCG treatment, because all seven patients in the control group who received hMG-hCG therapy or controlled ovarian stimulation of the IVF-ET protocol did not show improvement of RA blood flow or endometrial thickness.

Vitamin E produced 20% pregnancy rates in this study. Taking into consideration that most cases of patients with a thin endometrium do not show spontaneous improvement of the endometrial growth, vitamin E can be a useful treatment for patients with a thin endometrium. In addition to vitamin E, sildenafil citrate (Viagra) showed the greatest effect on RA-RI, endometrial thickness, and pregnancy rate and appears to have some promise for increasing pregnancy rate. There were no complaints of side effects from the patients in this study, although sildenafil citrate is known to cause side effects such as hypotension and headaches (24, 25). The use of intravaginal sildenafil suppositories made it possible to decrease the incidence of these side effects.

In conclusion, treatments that improve uterine RA blood flow seem to be quite beneficial to improve endometrial growth in patients with a thin endometrium. However, the present study is a pilot study with small number of subjects. A prospective randomized controlled trial with larger

samples is needed to demonstrate the efficacy of these treatments for thin endometrium.

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