Clinical Significance of Transvaginal Color Doppler Ultrasonography of the Ovarian Artery as a Predictor of Ovarian Response in Controlled Ovarian Hyperstimulation for In Vitro Fertilization and Embryo Transfer¹

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Purpose: Our purpose was to verify whether the blood flow impedance of the ovarian stromal artery in transvaginal color Doppler ultrasonography can predict the in vitro fertilization and embryo transfer (IVF-ET) outcomes.

Methods: Transvaginal color Doppler ultrasonographic examinations were performed in 99 patients undergoing controlled ovarian hyperstimulation (COH) for IVF-ET. The pulsatility index (PI) was evaluated in the bilateral ovarian stromal arteries on the starting day of COH (PI₁) and the day of hCG administration (PI₂). The patients were classified into three groups by the mean PI₁, PI₂, and Δ PI (PI₁ - PI₂), respectively, and the IVF-ET outcomes were analyzed and compared.

Results: There were no significant differences in the duration of COH, the total dosage of gonadotropins used for COH, the serum E₂ concentration on day of hCG administration, the number of follicles on the day of hCG administration, the number of oocytes retrieved or fertilized in vitro, and the number of embryos transferred between their respective PI₁, PI₂, and Δ PI values. However, the pregnancy rate was significantly lower in the higher PI₁ and PI₂ groups than in the lower groups ($p < 0.05$, $p < 0.05$). Significant positive correlations were also found between PI₁ and PI₂ and the total dosage of gonadotropins for COH ($Y = 0.483X + 27.1$, $r = 0.052$, $p < 0.05$; $Y = 0.877X + 26.6$, $r = 0.075$, $p < 0.05$).

Conclusions: Our results showed that the lower PI in the ovarian stromal artery during COH is associated with the higher the pregnancy rate and the smaller amount of gonadotropins used for effective COH. This study suggests that the color Doppler ultrasonographic index (PI) of the ovarian stromal artery during COH may be useful for predicting the success of IVF-ET in infertile patients.

KEY WORDS: Color Doppler ultrasonography; COH; IVF-ET; ovary; stromal artery.

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INTRODUCTION

Great advances have been made in the treatment of infertility, especially controlled ovarian hyperstimulation (COH) for in vitro fertilization and embryo transfer (IVF-ET) due to introduction of the GnRH agonist and the development of new gonadotropins. In determining the success of IVF-ET, the most important factors are good response of the ovaries to gonadotropin during COH and an adequate number of good quality embryos (1,2).

Doppler ultrasonography is a simple and noninvasive method and has recently been used to assess uterine or ovarian blood flow in normal cycling women (3-9). In addition, there were attempts to evaluate the endometrial receptivity and to predict pregnancy by measuring the Doppler indices of the ovarian and uterine artery (10). However, it is still controversial whether the Doppler indices of the ovarian artery, measured on day of oocyte retrieval or the day of embryo transfer, in pregnant women are different from those in nonpregnant women undergoing IVF-ET.

There have been several studies on the changes in ovarian artery blood flow during IVF-ET. However, the clinical significance of its Doppler index is yet to be determined (11-14). This study aimed to measure pulsatility index (PI) of the ovarian stromal arteries, analyze the COH results and the pregnancy outcome, and assess the efficacy of the PI of the ovarian stromal arteries in predicting the ovarian response and prognosis of the corresponding IVF-ET cycles.

MATERIALS AND METHODS

Patients and IVF Protocols

A total of 99 infertile patients who underwent IVF treatment for infertility between April 1999 and December 1999 were recruited in this study. Patients were included if they were in good physical and mental health, and at least 20 and at most 38 years of age at the time of enrollment. Women were excluded if their infertility was caused by endocrine abnormalities such as hyperprolactinemia, polycystic ovarian syndrome, or absence of ovarian function (baseline FSH > 18 mIU/mL). Women who had abused alcohol or drugs in the previous 12 months or women to whom investigational drugs had been administered within 3 months prior to enrollment were not allowed to enter the study. Controlled ovarian hyperstimulation was undertaken using subcutaneous triptorelin (Decapeptyl®; Ferring, Sweden) in a long protocol

combined with FSH (Metrodin HP®; Serono, Switzerland). Treatment with triptorelin (0.1 mg/day) began on Day 21 of the preceding cycle and continued until the day of hCG treatment. Treatment with 2-4 ampules of FSH, depending on the patients' previous or anticipated response, was initiated on Day 3 of the menstrual cycle. The treatment was then individualized in a step-down fashion. When the leading follicle reached 18 mm in mean diameter with a serum estradiol level of 200 pg/mL per mature follicle, 10,000 U of hCG (Profasi®; Serono, Switzerland) was administered. Oocyte retrieval was performed 36 h after the hCG injection. The ICSI (intracytoplasmic sperm injection) was performed according to conventional protocols and only on the MII oocytes. Up to four embryos were transferred vaginally on the second or third day after retrieval. The luteal phase was supported by i.m. progesterone.

Color Doppler Ultrasonography

Transvaginal color Doppler ultrasonographic examinations were performed using a Combison 530® (Medison, Korea) equipped with a 5.5 MHz transvaginal probe. The pulsatility index (PI) was measured in the bilateral ovarian stromal arteries on the starting day of COH (PI₁) (Fig. 1) and the day of hCG administration (PI₂) (Fig. 2). Examinations were performed between 8:00 A.M. and 10:00 A.M. with the patient in the lithotomy position. The arteries within ovarian stroma were visualized with the color Doppler techniques, and the blood flow waveforms were obtained by placing the Doppler gate over the colored areas and activating the pulsed Doppler function. The angle between the Doppler beam and the vessel was set to be close to 0°. All examinations were repeated three times by one investigator, and the Doppler indices were automatically calculated by the built-in computer when at least four consecutive uniform waveforms were obtained.

Study Design and Statistical Analysis

The patients were classified into three groups by the mean PI₁, PI₂, and Δ PI (PI₁ - PI₂), respectively, and the IVF-ET outcomes were analyzed and compared. The cutoff values for each variable (PI₁, PI₂, and Δ PI) were determined by calculating the mean and standard deviation of the PIs in each group. All data were analyzed by SPSS 7.5. ANOVA and chi-square tests were employed to assess the difference in COH outcome variables among the three groups.

Fig. 1. Representative photograph of the blood flow velocity waveforms of the ovarian stromal artery prior to the administration of gonadotropins for COH (A, PI = 2.53), and the corresponding B-mode image of the unstimulated ovary (B).

Fig. 2. Blood flow velocity waveforms of the ovarian stromal artery in the same patient on the day of hCG administration (A, PI = 2.03), and the corresponding B-mode image of the adequately stimulated ovary (B). The PI reduced from 2.53 (Fig. 1) on the gonadotropin starting day to 2.03 on the day of hCG administration.

Table I. Comparison of Clinical Characteristics Between Pregnant and Nonpregnant Groups

Characteristics	Pregnant (<i>n</i> = 50)	Nonpregnant (<i>n</i> = 49)
Age of patients (years)	33.3 ± 4.7	32.5 ± 4.2
Age of husbands (years)	35.4 ± 5.9	34.7 ± 3.8
Parity	0.28 ± 0.64	0.12 ± 0.39
Body mass index (BMI) (kg/m ²)	21.3 ± 2.5	21.1 ± 2.2
No. of previous IVF–ET cycles	2.3 ± 1.7	1.9 ± 1.5
Duration of infertility (months)	51.9 ± 42.5	60.0 ± 44.4
Causes of infertility		
Tubal	14	11
Peritoneal	10	12
Ovulatory	8	7
Male	11	12
Unexplained	4	3
Others	3	4

Note. Numbers are mean ± SD.

A pearson's correlation test and logistic regression analysis were used to evaluate the correlation between the PI and the outcome variables and the predictability of pregnancy by the PI values. $p < 0.05$ was regarded as statistically significant.

RESULTS

There was no significant difference in the clinical characteristics of the pregnant ($n = 50$) and nonpregnant ($n = 49$) groups (Table I). The average age of the pregnant group was 33.3 ± 4.7 years (mean ± SD), and the nonpregnant group was 32.5 ± 4.2 years. The duration of infertility was 51.9 ± 42.5 months for the pregnant group and 60.0 ± 44.4 months for the nonpregnant group. The number of previous attempts was 2.3 ± 1.7 cycles for the pregnant group and 1.9 ± 1.5 cycles for the nonpregnant group. There was no

difference in the distribution of infertility factors, husband's age, parity, and body mass index (BMI).

The PI₁, measured immediately before the start of COH, was 1.75 ± 0.85 in the right ovarian stromal artery, 1.85 ± 1.05 in the left side, and the average of these two values was 1.80 ± 0.94 . The PI₂, measured on the day of hCG injection, was 1.44 ± 0.69 in the right side, and 1.57 ± 0.82 in the left, which averaged as 1.80 ± 0.94 . The change in PI during COH, Δ PI (PI₁ – PI₂), was 0.31 ± 0.22 in the right ovarian stromal artery and 0.28 ± 0.24 in the left. The average value was 0.29 ± 0.22 .

The patients were divided into three groups according to the cutoff values based on $0.5 \times$ SD from the mean value, and compared with the IVF outcomes. The cutoff values were 1.3 and 2.3 for the PI₁, 1.0 and 2.0 for the PI₂, and 0.2 and 0.4 for the Δ PI, respectively.

There was no difference among the groups with a PI₁ < 1.3 (Group A: $n = 44$), $1.3 \leq$ PI₁ < 2.3 (Group B: $n = 22$), and a PI₁ $\geq$ 2.3 (Group C: $n = 33$), in IVF outcomes such as COH duration, the total gonadotropin dose used, the serum E₂ concentrations and the number of follicles $\geq$ 14 mm on the day of hCG administration, the number of oocytes retrieved and embryos transferred, and the fertilization rate (Table II). However, the pregnancy rate was 61.4% (27/44) for Group A, 45.5% (10/22) for Group B, and 33.3% (11/33) for Group C, respectively. Group A showed a higher pregnancy rate than Group C ($p < 0.05$) (Table II).

On the day of hCG administration, the PI₂ did not demonstrate any significant difference in the above outcome variables among the three groups; PI₂ < 1.0 (Group D: $n = 41$), $1.0 \leq$ PI₂ < 2.0 (Group E: $n = 26$), and PI₂ $\geq$ 2.0 (Group F: $n = 32$), either.

Table II. Comparison of IVF–ET Outcomes by Mean PI₁ of Ovarian Stromal Artery on Starting Day of COH

No.	PI ₁ ^a < 1.3 (<i>n</i> = 44)	$1.3 \leq$ PI ₁ < 2.3 (<i>n</i> = 22)	PI ₁ $\geq$ 2.3 (<i>n</i> = 33)
Duration of COH ^b (days)	9.7 ± 2.2	10.1 ± 2.3	9.7 ± 1.7
Gonadotropins administered (ampules)	27.8 ± 9.5	27.3 ± 9.1	28.5 ± 8.3
Serum E ₂ on hCG day (pg/mL)	2174.5 ± 1887.4	1931.7 ± 1417.4	2185.2 ± 1429.9
Follicles	12.5 ± 7.4	13.5 ± 7.1	13.9 ± 5.7
Follicles with diameter $\geq$ 14 mm	8.1 ± 3.5	9.6 ± 3.6	10.0 ± 3.6
Oocytes retrieved	9.3 ± 5.3	10.0 ± 6.1	10.8 ± 5.5
Oocytes fertilized	5.8 ± 3.9	6.0 ± 4.2	6.2 ± 3.3
Embryos transferred	3.8 ± 1.5	3.6 ± 1.4	4.3 ± 1.6
Pregnancy rate (%)	61.4 (27/44) ^c	45.5 (10/22)	33.3 (11/33) ^c

Note. Numbers are mean ± SD.

^a Pulsatility index on starting day of COH.

^b Controlled ovarian hyperstimulation.

^c $p < 0.05$.

Table III. Comparison of IVF–ET Outcomes by Mean PI₂ of Ovarian Stromal Artery on hCG Day of COH

No.	PI ₂ ^a < 1.0 (<i>n</i> = 41)	1.0 ≤ PI ₂ < 2.0 (<i>n</i> = 26)	PI ₂ ≥ 2.0 (<i>n</i> = 32)
Duration of COH ^b (days)	9.8 ± 2.2	9.9 ± 2.3	9.7 ± 1.7
Gonadotropins administered (ampules)	28.1 ± 9.7	26.9 ± 8.7	28.5 ± 8.3
Serum E ₂ on hCG day (pg/mL)	2202.0 ± 1941.3	1860.3 ± 1364.1	2241.3 ± 1417.2
Follicles	12.8 ± 7.1	13.1 ± 7.2	13.8 ± 5.7
Follicles with diameter ≥ 14 mm	8.0 ± 3.4	9.6 ± 3.5	9.5 ± 4.0
Oocytes retrieved	9.2 ± 5.3	9.9 ± 5.8	10.0 ± 3.6
Oocytes fertilized	5.8 ± 3.9	5.8 ± 4.1	6.3 ± 3.3
Embryos transferred	3.8 ± 1.5	3.5 ± 1.4	4.3 ± 1.6
Pregnancy rate (%)	61.0 (25/41) ^c	46.2 (12/26)	34.3 (11/32) ^c

Note. Numbers are mean ± SD.

^a Pulsatility index on hCG day of COH.

^b Controlled ovarian hyperstimulation.

^c *p* < 0.05.

However, the pregnancy rate was 61.0% (25/41) for Group D, 46.2% (12/26) for Group E, and 34.3% (11/32) for Group F, respectively. Group D showed a higher pregnancy rate compared to Group F (*p* < 0.05) (Table III).

Furthermore, the ΔPI did not affect the IVF outcome as did the PI₁ or the PI₂ among the three groups; ΔPI < 0.2 (Group G: *n* = 40), 0.2 ≤ ΔPI < 0.4 (Group H: *n* = 15), and ΔPI ≥ 0.4 (Group I: *n* = 44). However, Group G showed a higher pregnancy rate than Group I (*p* < 0.05); 60.0% (24/40) for Group G, 60.0% (9/15) for Group H, and 34.1% (15/44) for Group I, respectively (Table IV).

PI₁, PI₂, and ΔPI did not show any correlation with any outcome variables except for the total dose of gonadotropins used. More gonadotropins were required the higher PI₁ or PI₂ ($Y = 0.483X + 27.1$, *r* = 0.052, *p* < 0.05; $Y = 0.877X + 26.6$, *r* = 0.075, *p* < 0.05, respectively) (Figs. 3 and 4). Significant negative correlations were also found between ΔPI and the total dosage of gonadotropins used for COH

($Y = -1.628X + 28.4$, *r* = 0.039, *p* < 0.05) (data not shown).

DISCUSSION

Numerous studies have been performed to improve the clinical efficacy of assisted reproductive technology (ART) including IVF–ET and to predict the prognosis of the corresponding cycles. One of the most important factors in achieving IVF success is the number and quality of oocytes retrieved (1,2). In this context, Doppler ultrasound was introduced into the field of gynecology to assess the uterine or ovarian blood flow during the menstrual cycle (3–9), and many studies have attempted to evaluate ovarian circulation during hyperstimulation (11–14).

This study sought to determine whether quantitative analysis of the ovarian blood flow during COH predicts the prognosis of the IVF cycles. The ovarian stromal artery was used for measuring ovarian

Table IV. Comparison of IVF–ET Outcomes by Change in Mean ΔPI of Ovarian Stromal Artery During COH

No.	ΔPI ^a < 0.2 (<i>n</i> = 40)	0.2 ≤ ΔPI < 0.4 (<i>n</i> = 15)	ΔPI ≥ 0.4 (<i>n</i> = 44)
Duration of COH ^b (days)	9.8 ± 2.2	9.9 ± 2.1	9.7 ± 1.9
Gonadotropins administered (ampules)	28.3 ± 9.8	29.1 ± 8.9	27.2 ± 8.3
Serum E ₂ on hCG day (pg/mL)	2204.4 ± 1966.6	1712.4 ± 1198.0	2181.5 ± 1429.2
Follicles	12.8 ± 7.2	12.5 ± 6.6	13.8 ± 6.2
Follicles with diameter ≥ 14 mm	7.9 ± 3.4	9.1 ± 3.5	10.1 ± 3.6
Oocytes retrieved	9.1 ± 5.3	8.5 ± 3.7	11.3 ± 5.9
Oocytes fertilized	5.7 ± 3.9	6.0 ± 3.3	6.2 ± 3.8
Embryos transferred	3.8 ± 1.5	3.9 ± 1.5	4.0 ± 1.5
Pregnancy rate (%)	60.0 (24/40) ^c	60.0 (9/15)	34.1 (15/44) ^c

Note. Numbers are mean ± SD.

^a Pulsatility index on starting day of COH (PI₁) – Pulsatility index on hCG day (PI₂).

^b Controlled ovarian hyperstimulation.

^c *p* < 0.05.

Fig. 3. A positive correlation was demonstrated between the mean PI of the ovarian stromal artery on starting day of COH and the total dosage of gonadotropins administered for COH.

circulation, the PI was assessed on the initiation of COH (PI₁) and hCG injection (PI₂). The measured values were compared and analyzed after the patients were divided into three groups by each mean and SD.

There was no difference in clinical characteristics between the 50 pregnant and 49 nonpregnant patients, including age and BMI, which can affect ovarian circulation, and age of their husbands, parity, duration of infertility, and the number of previous cycles that may influence the IVF-ET prognosis. Concomitant medication and past history of pelvic surgery can also affect ovarian blood flow. In this study, there was no patient who was on medications that may alter ovarian circulation. Pirhonen and Pulkkinen (15) reported that drugs such as nimessulide or naproxen did not cause any change in PI of the ovarian branch of the uterine artery. Even with the possibility of not reporting the patients' medication status (e.g., non-steroid antiinflammatory drugs for dysmenorrhea), it might not have a major impact on the Doppler index. In terms of surgical history, Geber and Caetano (16) reported no significant difference in the PI of ovarian artery before and after a tubal ligation. In

contrast, La Torre *et al.* (17) demonstrated that the PI of the ovarian artery was lower after conservative laparoscopic surgery of the ovarian endometrioma. This investigation included five patients in the pregnant group and six in the nonpregnant group who had received tubal ligation; three in the pregnant group and four in the nonpregnant group who had undergone laparoscopic surgery for endometriosis, which did not influence the study results.

There has been a controversy over reproducibility of the Doppler measurement due to intra- and interobserver variance. Farquhar *et al.* (18) questioned the clinical efficacy of measuring the PI in the pelvic vessels because of interobserver technical variability. In addition, Zaidi *et al.* (19) maintained that the PI of the ovarian stromal artery in healthy women was high in the morning and nadir in the afternoon due to the circadian fluctuation. However, other investigators asserted that the PI and RI measurements in the intraovarian arteries were reproducible, while the inconsistency observed in the velocity measurements in the intraovarian arteries raises some doubt as to the reliability of these

Fig. 4. A positive correlation was demonstrated between the mean PI of the ovarian stromal artery on the day of hCG administration and the total dosage of gonadotropins administered for COH.

measurements (9). Consequently, this investigation was done by a single observer in order to eliminate interobserver variance, and the examinations were done from 8:00 A.M. to 10:00 A.M. to minimize the circadian variation in the PI. However, the possibility that the data obtained by a single observer could not be confirmed by another observer cannot be negated, and it would somewhat limit the reliability of the reported results.

This study showed that the higher the PI of the ovarian stromal artery was associated with, the lower the pregnancy rate of the corresponding IVF cycle, on the start day of COH and on the day of hCG injection, respectively. These results are considered to be due to the availability of good-quality oocytes and embryos by the better circulation in the ovarian stromal artery. Consequently, the supply of oxygen, nutrients, hormones, and growth factors is improved when the PI of ovarian stromal artery is lower. However, the significance of the PI in ovarian circulation is still controversial. Tinkanen *et al.* (20) reported that infertility

patients, with the male factor patients not included, had higher PIs in the ovarian arteries than those of the control group in the luteal phase. Engmann *et al.* (10) compared the PIs of the ovarian stromal artery between the pregnant and nonpregnant groups on the day of hCG administration of the COH, and reported that the PI was lower in the pregnant group. In contrast, Tekay and Jouppila (9) and Bhal *et al.* (14) reported that there was no significant difference in the PI of the ovarian stromal arteries between the pregnant and nonpregnant groups of the IVF cycles. This controversy probably resulted from the fact that the Doppler indices of the ovarian circulation cannot precisely predict the pregnancy outcome of the corresponding IVF cycle. The contribution of the genetic makeup of embryos and the endometrial receptivity is also very important for achieving a successful pregnancy besides the adequate ovarian circulation.

The PIs of the ovarian and ovarian stromal arteries are known to decrease according to the follicular development, both in the natural (3–6) and COH cycles

(11,12). Data from this study is consistent with previous reports and the PI of the ovarian stromal artery was lower on the day of hCG injection than on the start day of COH.

The greater the decrease in PI during COH (Δ PI), the higher the increase in the intraovarian blood flow was expected to be and the higher the pregnancy rate of the corresponding IVF cycles. However, our data suggests that the pregnancy rate of the group with a Δ PI of 0.4 or higher (Group I) was significantly lower. In regard to the relation between PI_1 and Δ PI, the PI_1 of the group with a Δ PI of 0.4 or higher (Group I) was significantly higher than that of the group with a Δ PI of between 0.2 and 0.4 (Group H) or that of the group with a Δ PI < 0.2 (Group G) (2.56 ± 0.70 vs. 2.06 ± 0.64 vs. 0.87 ± 0.12 ; $p < 0.001$). A similar trend was observed for the PI_2 . The PI_2 of the group with a Δ PI of 0.4 or higher (Group I) was significantly higher than that of the group with a Δ PI less than 0.2 (Group G) (2.06 ± 0.65 vs. 0.82 ± 0.11 ; $p < 0.01$). This suggests that the pregnancy rate was more influenced by the PI_1 or the PI_2 than the Δ PI. As shown in Tables II and III, the higher the PI_1 or PI_2 value, the lower the pregnancy rate.

There were a few reports regarding the correlation between the Doppler indices and the endocrine variables or the COH outcomes. Hata *et al.* (6) investigated the serum progesterone level in women with normal menstrual cycles, and reported that the PI of the ovarian stromal artery correlated with the serum progesterone concentration but not with the estradiol levels. Weiner *et al.* (11) reported that, in infertile patients undergoing COH, there was a significant correlation between the PI of the ovarian stromal artery and both the number of follicles 15 mm or larger and the serum estradiol level on the day of hCG administration. This could not be demonstrated in women showing a poor response to COH. In this study, there was no significant correlation between any Doppler index investigated and either the serum estradiol concentration or the number of follicles with a diameter of 14 mm or larger on the day of hCG administration. However, the lower the PI_1 or PI_2 and the higher the Δ PI, the smaller is the amount of gonadotropins required for a successful COH (Figs. 3 and 4). If the absolute value of and the change in PI during COH could adequately reflect the ovarian circulation, these results may imply that the better the ovarian circulation, the smaller the amount of gonadotropins necessary for an effective COH.

The possibility that the cell stage and embryo quality besides the number of transferred embryos would

influence the results of IVF was considered. The cumulative embryo score (CES) was calculated by the product of number of blastomeres, embryo grade, and number of embryos transferred. The CES showed no difference among the three PI groups both on COH starting day (49.9 ± 4.9 vs. 48.0 ± 9.3 vs. 49.0 ± 5.8) and hCG day (50.1 ± 5.0 vs. 49.4 ± 9.3 vs. 47.5 ± 5.8).

The success of IVF cycles is influenced by both ovarian and uterine factors. Even though the number and quality of oocytes and embryos are good, pregnancy rate should be lower if the uterine receptivity is poor. In this study, ovarian Doppler indices were studied, however, further studies are necessary to coevaluate the ovarian Doppler indices and the uterine parameters. Since the Doppler indices of uterine artery are not reported to adequately reflect the uterine receptivity (21,22), other uterine factors would be more appropriate for the evaluation. Our preliminary data also revealed that the PI of uterine artery showed no difference between the pregnant and nonpregnant groups on the day of hCG administration (2.41 ± 0.47 vs. 2.70 ± 0.61). In addition, the investigations will be required to analyze which factor has higher impact on the results of IVF cycles among functional parameters, such as Doppler indices, and morphological parameters, such as ovarian volume or antral follicle count.

The authors measured the Doppler indices of the ovarian stromal arteries in women undergoing COH for IVF-ET using transvaginal Doppler ultrasound. These were compared and analyzed according to the COH outcomes in the corresponding IVF cycles. This study showed that the higher the PI of the ovarian stromal artery is associated with, the lower the pregnancy rate and the larger the amount of gonadotropins that were required. In conclusion, the color Doppler ultrasonographic index (PI) of the ovarian stromal artery during COH may be useful for predicting the success of IVF-ET in infertile patients.

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