Ovarian Stromal Blood Flow Following Clomiphene Citrate Challenge Test in Infertile Women

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ABSTRACT: *Purpose.* To compare ovarian stromal blood flow indices in the follicular phase and after clomiphene citrate (CC) in infertile women.

Methods. Pulsatility index (PI), resistance index (RI), and peak systolic blood flow velocity (PSV) of ovarian stromal vessels were determined by spectral Doppler analysis in the early follicular phase and on day 10 after CC. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol concentrations were determined.

Results. A total of 69 infertile women were included in the analysis. No significant differences in the average PI, RI, and PSV of ovarian stromal blood flow were demonstrated in the follicular phase and after CC despite a significant increase in serum estradiol concentration after CC. Serum FSH concentration was similar in the follicular phase and after CC, while serum LH concentration was significantly higher after CC. In the right ovary, ovarian stromal blood flow was absent in 13 (18.8%) patients in the follicular phase and in 6 (8.7%) patients after CC, but the difference did not reach statistical significance. In the left ovary, ovarian stromal blood flow was absent in 13 (18.8%) and 12 (17.4%) patients in the follicular phase and after CC, respectively.

Conclusion. Ovarian stromal blood flow indices were similar in the follicular phase and after CC. © 2008 Wiley Periodicals, Inc. *J Clin Ultrasound* **36**:403–408, 2008; Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jcu.20443

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The development of multiple follicles in response to gonadotropin stimulation is the key factor leading to a successful outcome of in vitro fertilization (IVF). Poor ovarian response may be associated with poor pregnancy rates, and many of these cycles are canceled without proceeding to oocyte retrieval.[1] On the other hand, exaggerated ovarian response leads to an increased risk of ovarian hyperstimulation syndrome,[2] and the resulting high serum estradiol (E2) concentrations may adversely affect the outcomes of IVF.[3] The prediction of ovarian responses prior to stimulation is useful in counseling patients and may be helpful in tailoring the dosage of gonadotropin to individual patients.

Sonography is essential during IVF treatment for monitoring ovarian response, assessing endometrial receptivity, and guiding the transvaginal aspiration of oocytes and subsequent transcervical transfer of embryos to the uterus. Sonographic parameters have been examined to predict the ovarian response to gonadotropins, including ovarian volume,[4] antral follicle count,[5] and ovarian stromal blood flow.[6–10] Folliculogenesis in the human ovary is a complex process regulated by a variety of endocrine and paracrine signals.[11] The availability of an adequate vascular

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supply to provide endocrine and paracrine signals may play a key role in the regulation of follicle growth.[12] It is postulated that increased ovarian stromal blood flow may lead to a greater delivery of gonadotropins to the granulosa cells of the developing follicles. Ovarian stromal blood flow can be assessed by color or power Doppler sonography. Power Doppler uses the amplitude of the Doppler signal as opposed to the mean frequency shift, is more sensitive to lower velocities than color Doppler, and is essentially angle-independent.[13]

Early follicle-stimulating hormone (FSH) concentration is widely used in many IVF units to predict ovarian response.[14] To identify a greater number of women with decreased ovarian reserve, challenging the pituitary gland may be more likely to uncover an abnormality that would be missed by obtaining basal FSH concentration alone. Navot et al[15] first described the clomiphene citrate challenge test (CCCT), which consisted of measuring serum FSH concentrations on cycle day 3 and then again on day 10 after administration of 100 mg of clomiphene citrate (CC) from day 5 to day 9.

Because the use of CC stimulates the growth of antral follicles, we postulated that basal ovarian stromal blood flow would be increased after CCCT. The objective of this prospective study was to compare ovarian stromal blood flow indices measured by spectral Doppler analysis in the early follicular phase and after CCCT.

MATERIALS AND METHODS

Women who were >40 years old at the time of the treatment or whose basal FSH concentration upon repeated testing on recruitment was ≥10 IU/l were advised against IVF according to the recruitment guideline issued by the Hospital Authority in Hong Kong. Women who consecutively visited the Department of Obstetrics and Gynaecology of the University of Hong Kong between January 2002 and August 2002 for their first IVF treatment were recruited for the study when the following criteria were met: (1) no history of ovarian surgery and (2) no steroid treatment within 6 months before IVF. Poor visualization of the ovaries because of abdominal position, the presence of an ovarian cyst ≥20 mm in diameter, and the presence of polycystic ovaries on scanning[16] were the reasons for retrospective exclusion. Each patient gave written informed consent prior to participating in the study, which was approved by the Ethics Committee, Faculty of Medicine of the University of Hong Kong.

Two months prior to the treatment cycle, they visited the clinic for a transvaginal sonographic examination and a blood test for serum FSH, luteinizing hormone (LH), and E2 concentrations in the early follicular phase (days 2–4) of the cycle. They were asked to take CC (Clomid; Sanofi-Avertis, Bridgewater, NJ) 100 mg daily from day 5 to day 9. Transvaginal scanning and blood test were repeated on day 10.

Transvaginal scanning was performed at around 8:00–10:00 A.M. using an SSD-5500 scanner and a 6.5-MHz endovaginal probe (Aloka, Tokyo, Japan) with the same sonographic setting after the patients had emptied the bladder. The setting condition for the power Doppler mode was as follows: power flow gain, 16 dB; flow filter, 3; color line density, B&W high, color medium, and PRF −6.22. All these patients were evaluated by two-dimensional sonography only. The number of antral follicles measuring <10 mm in diameter was counted in the early follicular phase,[4] and AFC was the sum of antral follicles on both sides. The presence or absence of ovarian stromal blood flow was determined using power Doppler sonography. Flow velocity waveforms were obtained via spectral Doppler analysis from stromal blood vessels away from the ovarian capsule and a growing follicle, if present. The "gate" of the Doppler analysis was positioned where a vessel with good color signals was identified on the screen. The pulsatility index (PI), resistance index (RI), and peak systolic blood flow velocity (PSV) of stromal vessels was calculated electronically when 3 similar, consecutive waveforms of good quality were obtained. Ovarian stromal blood flow was evaluated at 3 positions at random, and the one with the highest PSV was chosen. When stromal flow was shown on both ovaries, the mean value was used, because there were no significant differences in PI, RI, and PSV between the left and right sides. In the case of ovarian flow on 1 ovary only, Doppler flow indices of that side were taken. Patients with no ovarian blood flow identified on either side were excluded from the calculation of Doppler indices. The intraobserver coefficient of variation was 9.6% for PI, 4.1% for RI, and 16% for PSV.

The blood samples were processed by centrifugation within two hours of collection, and the supernatant was stored at −20°C for subsequent analysis. Serum FSH, LH, and E2 concentrations were measured using commercially available kits (Automated Chemiluminescence ACS-180 System; Bayer Corporation, Tarrytown, NY). The

TABLE 1
Comparison of Hormonal Parameters and Ovarian Stromal Blood Flow Indices in the Early Follicular Phase and after CCCT

	Early Follicular Phase (n = 69)	After CCCT (n = 69)	*p* Value
Average PI*	1.17 (0.46–4.62)	1.14 (0.67–3.63)	>0.05
Average RI*	0.66 (0.31–1.94)	0.68 (0.49 – 1.63)	>0.05
Average PSV† (cm/second)	12.9 ± 4.2	12.3 ± 4.1	>0.05
Serum FSH* (IU/l)	6.9 (3.3–19.9)	7.3 (2.6–43.1)	>0.05
Serum LH* (IU/l)	3.9 (1.7–10.0)	9.1 (2.2–21.0)	<0.001
Serum E2* (pmol/l)	75.0 (37–547)	1221.0 (37–3583)	<0.001

* Results given as the median (range).
†Results given as the mean ± SD.

TABLE 2
Comparison of Hormonal Parameters and Ovarian Stromal Blood Flow Indices in the Early Follicular Phase and after CCCT, after the Exclusion of Those Showing No Ovulatory Response

	Early Follicular Phase (n = 62)	After CCCT (n = 62)	*p* Value
Average PI*	1.19 (0.46–4.62)	1.14 (0.67–3.63)	>0.05
Average RI*	0.66 (0.31–1.94)	0.69 (0.49 – 1.63)	>0.05
Average PSV† (cm/second)	12.1 ± 3.7	12.0 ± 4.3	>0.05
Serum FSH* (IU/l)	6.8 (3.3–15.7)	7.4 (2.6–17.0)	>0.05
Serum LH* (IU/l)	3.9 (1.8–9.2)	9.2 (4.0–21.6)	<0.001
Serum E2* (pmol/l)	72.0 (37–547)	1254.0 (333–3583)	<0.001

* Results given as the median (range).
†Results given as the mean ± SD.

FSH assay is standardized against the World Health Organiziation 2nd International Standard 94/632 reference material. The sensitivity of the FSH assay was 0.3 pmol/l, and the intra- and interassay coefficients of variation were 2.8% and 4.6%, respectively. The sensitivity of the LH assay was 0.07 IU/L, and the intra- and interassay coefficients of variation were 4.5% and 5.2%, respectively. The sensitivity of the E2 assay was 36.7 pmol/l, and the intra- and interassay coefficients of variation were 8.1% and 8.7%, respectively.

The primary outcome measures included averaged PI, RI, and PSV of ovarian stromal blood flow. Continuous variables were given as the mean ± SD if normally distributed, or as the median (range) if not normally distributed. Statistical tests were performed using a paired *t* test or Wilcoxon signed-rank test as appropriate. Statistical analysis was performed using the SPSS version 12.0 (SPSS Inc., Chicago, IL). A 2-tailed value of $p < 0.05$ was considered statistically significant.

RESULTS

A total of 90 consecutive eligible patients who passed the screening process were scanned. Twenty-one patients were excluded from the study because of poor visualization of the ovaries (4 patients), presence of an ovarian cyst in the early follicular phase (10 patients), and the presence of polycystic ovaries (7 patients). Therefore, 69 women were included in the final analysis: 23 (33.3%) with tubal factors, 7 (10.1%) with endometriosis, 35 (50.7%) with male infertility, 3 (4.4%) with no explanation, and 1 (1.5%) with mixed causes. The mean ± SD age of the women was 35.0 ± 3.2 years, and the median duration of infertility was 5.0 (range, 2–16) years. Forty-eight (69.6%) women had primary infertility, while eight (11.6%) were smokers. The mean ± SD antral follilcular count (AFC) was 10.3 ± 4.9.

No significant differences in the averaged PI, RI, and PSV of ovarian stromal blood flow were demonstrated in the follicular phase and after CCCT despite a significant increase in serum E2 concentration after CC (Table 1). The serum FSH concentration was similar in the follicular phase and after CCCT, whereas serum LH concentration was significantly higher after CCCT. In the right ovary, ovarian stromal blood flow was absent in 13 (18.8%) patients in the follicular phase and in 6 (8.7%) patients after CCCT, but the difference did not reach statistical significance ($p > 0.05$). In the left ovary, ovarian stromal blood flow was absent in 13 (18.8%) and 12 (17.4%) patients in the follicular phase and after CCCT, respectively.

Serum E2 remained low (<200 pmol/l) after CCCT in 7 (10.1%) patients. After excluding these 7 patients who had no response to CC, there were again no significant differences in the average PI, RI, and PSV of ovarian stromal blood flow (Table 2).

DISCUSSION

In the present study, we compared the ovarian stromal blood flow indices of infertile women in the early follicular phase and after CCCT while they were on a waiting list for their first IVF cycle. It is important to highlight that we examined a group of infertile women having relatively normal ovarian reserve, as suggested by their age of <40 years at the time of IVF and a basal FSH concentration of <10 IU/l. Patients who did not satisfy these criteria required self-funded treatment cycles in a private unit. We found no significant differences in the averaged ovarian stromal blood flow indices in the follicular phase and after CCCT despite a significant increase in serum E2 after CCCT.

Ovarian stromal blood flow has been examined in IVF treatment to predict the ovarian response to gonadotropins.[6–10] The mean ovarian stromal PSV before pituitary down-regulation was demonstrated to be significantly correlated with the number of follicles after controlling for patients' age.[6] Those with normal ovarian responses (>6 follicles at retrieval) had a significantly higher velocity than poor responders (10.2 ± 5.8 cm/second versus 5.2 ± 4.2 cm/second). Other Doppler flow indices were not useful. Similarly, ovarian stromal PSV after pituitary down-regulation was the most important single independent predictor of the number of oocytes obtained in patients with normal basal FSH concentration, when compared with the age of women, basal FSH concentration, E2 concentration, or FSH/LH ratio.[8] Bassil et al[7] reported that women with an ovarian blood flow RI of >0.56 had a significantly longer stimulation period and a significantly lower mean number of oocytes retrieved. Both Zaidi et al[6] and Engmann et al[8] also recruited patients with polycystic ovaries.

Using 3-dimensional sonography with power Doppler, Kupesic and Kurjak[9] demonstrated that AFC achieved the best predictive value for a favorable IVF outcome, followed by ovarian stromal flow index, E2 on human chorionic gonadotropin (hCG) administration day, total ovarian volume, total ovarian stromal area, and age. In 136 women undergoing the first IVF cycle, we evaluated the role of ovarian stromal blood flow in the prediction of ovarian response by comparing age, body mass index (BMI), basal FSH concentration, AFC, and ovarian stromal blood flow indices as measured via 2-dimensional power Doppler sonography.[10] In that study, we showed that basal FSH concentration achieved the best predictive value in relation to the number of oocytes obtained, followed by AFC and BMI. AFC was the only predictive factor of serum E2 concentration on the day of hCG, whereas BMI was predictive of the dosage of gonadotropin used. Therefore, ovarian stromal blood flow indices measured via power Doppler sonography had no predictive value for the number of oocytes obtained, serum E2 concentration on the day of hCG, and the duration and dosage of gonadotropin used.

Previous studies have shown that abnormal CCCT is predictive of poor ovarian response, cycle cancellation, and reduced pregnancy rate.[17–19] These studies evaluated stimulated FSH concentrations only. Because the use of CC stimulates the growth of follicles, we postulated that basal ovarian stromal blood flow would be increased as well after CCCT. The stimulated ovarian stromal blood flow may serve as a new sonographic parameter to predict ovarian response. However, this study could not demonstrate any significant change in ovarian stromal blood flow after CCCT. Subgroup analysis revealed the same finding after excluding 7 patients who showed no ovulatory response after CC (ie, serum E2 remained <200 pmol/l). The percentage of absent ovarian stromal blood flow in the right ovary seemed to be reduced after CCCT, but the difference was not statistically significant. The percentage of absent ovarian stromal blood flow in the left ovary remained similar in the follicular phase and after CCCT.

Although blood flow is increased around developing follicles in the course of follicular phase during ovarian stimulation,[20] ovarian stromal blood flow may not be increased after CC stimulation. The power Doppler examination was repeated on day 10 of the cycle, and there may not be any significant change in ovarian stromal blood flow in the early follicular phase. It may be explained by the specialized microcirculation of the ovary. We examined tortuous spiral arteries within the ovarian stroma, which undergo numerous divisions and decrease in caliber. The spiral course of these arteries enlarges the frictional surface area during blood flow, causing the increase of the vascular resistance and decrease

in blood pressure. Turbulence within the intrinsic ovarian system (due to the spiral course of these vessels) and angled branching pattern may help to maintain the stability of blood flow after CC.

Because some patients had multiple follicles developing on both sides after CC, we did not attempt to compare the ovarian stromal blood flow indices in the follicular phase and after CCCT on each side separately. It would be interesting to determine the ovarian stromal blood flow using 3-dimensional power Doppler sonography, which provides a unique tool with which to examine the ovarian stromal blood supply as a whole, as opposed to the analysis of small individual stromal vessels in 2D planes.[21]

The regulation of ovarian angiogenesis is a complex process involving many angiogenic factors.[22] Elevated serum LH concentration has been considered as a cause of increased ovarian stromal blood flow in women with polycystic ovaries through the action of prostaglandins. Aleem and Predanic[23] demonstrated a positive relationship between serum LH concentration and increased PSV of ovarian vessels. A linear increase in the capillary cross-sectional area of the theca interna was observed after the LH surge in a spontaneous cycle, and the increase in the capillary area was attributed to vasodilatation rather than the increase in the number of vessels.[24] Prostaglandins E2 and I2 are potent vasodilators that markedly increase local blood flow.[25] Serum LH concentration was significantly increased after CCCT. Based on our findings, serum LH concentration does not appear to have a role in the ovarian stromal flow of infertile women without polycystic ovaries.

In conclusion, the ovarian stromal blood flow indices measured via 2-dimensional power Doppler sonography were similar in the follicular phase and after CCCT.

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