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Assessment of Endometrial Perfusion with Doppler Ultrasound in Spontaneous and Stimulated Menstrual Cycles

Akihito Nakai, Akishige Yokota, Tatsuo Koshino and Tsutomu Araki

Department of Obstetrics and Gynecology, Nippon Medical School

Abstract

Objective: Endometrial perfusions were measured by Doppler ultrasound to evaluate the influence of spontaneous menstrual cycles and to study the effect of clomiphene citrate.

Methods: Flow waveforms in right and left uterine arteries were obtained by using transvaginal color Doppler ultrasonography in infertile women with 60 spontaneous menstrual cycles and 37 clomiphene citrate stimulated cycles from the follicular to the luteal phase.

Results: In the spontaneous menstrual cycles, the uterine arterial blood flow increased significantly from the follicular phase to the day of ovulation and then increased markedly to about 200～230% of the follicular phase after the 6 th day of ovulation. In the clomiphene citrate stimulated cycles, the uterine arterial blood flow did not change during the periovulatory period and then increased significantly to about 180～220% of the follicular phase after the 6 th day of ovulation.

Conclusions: In the present study, the clomiphene citrate stimulated cycles showed lower endometrial perfusion during the periovulatory period compared with those in the spontaneous menstrual cycles. The results suggest that the assessment of endometrial perfusion with Doppler ultrasound can be used to reveal unexplained infertility problems in induced ovarian cycles.
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Key words: ultrasonography, infertility, endometrial perfusion, clomiphene citrate

Introduction

The development of transvaginal color Doppler ultrasound has been providing the ability to evaluate the female reproductive system on an anatomical and physiological basis. This technique makes it possible to measure vascular impedance in both the uterine artery and the ovarian artery in infertile women[1–5].

Several investigators have attempted to evaluate uterine receptivity by Doppler ultrasound in the uterine artery at different stages of the menstrual cycle. Goswamy et al.[6] suggested that a high resistance to uterine blood flow in the luteal phase might be one of the causes of unexplained infertility. Our preliminary study[7] also demonstrated that the conception cycles showed lower vascular impedance in the uterus during the periovulatory period compared with those in the non-conception cycles. However, relatively little is known about changes in uterine perfusion during the periovulatory period of induced folliculogenesis.

In the present study, by using the transvaginal color Doppler sonography technique, we sought to assess whether there is considerable difference

Correspondence to Akihito Nakai, MD, Department of Obstetrics and Gynecology, Tama Nagayama Hospital, Nippon Medical School, 1–7–1 Nagayama, Tama-shi, Tokyo 206–8512, Japan
E-mail: nakai-3@nms.ac.jp
Journal Website (http://www.nms.ac.jp/jnms/)

between spontaneous and hormonally stimulated menstrual cycles in the uterine perfusion of infertile women.

Subjects and Methods

Transvaginal color Doppler was performed on 97 patients with unexplained infertility at Tama Nagayama Hospital of Nippon Medical School. All subjects provided written informed consent for participation in this study, which was approved by the institutional review board. The inclusion criteria were formulated to exclude obvious female and male causes of infertility. Accordingly, all the subjects had a history of regular menstrual cycles confirmed by temperature graph, a normal pelvis assessed by transvaginal sonography and patent fallopian tubes as determined by hysterosalpingography. The mean age of patients was 32 years (range 27～38), the mean length of their previous menstrual cycles was 29 days (range 27～32), and the mean infertility duration was 3.5 years (range 2～9). Eighty-five of the women were nulliparous and 12 had had a single pregnancy with an uncomplicated delivery at term.

The patients were randomly divided into two groups. Of these, 60 patients who had no medication were recruited as the spontaneous group, and the other 37 patients as the hormonally stimulated group. In all the patients in the hormonally stimulated group 100 mg of clomiphene citrate was prescribed to be taken orally, two times per day for 5 days, starting on the 5 th day of the period.

All studies were performed daily, starting on the 5 th day from the onset of menstruation and finishing on the 12 th day after ovulation. Using B-mode transvaginal sonography, endometrial thickness of the uterus and leading follicle of the ovary were explored. The maximum thickness of the endometrium was measured in the longitudinal plane of the uterus. The measurement included the echogenic interface between the endometrium and myometrium, but excluded the hypo-echogenic area[4,8]. Ovulation was defined by transvaginal sonography as a decrease in follicular size of ≤ 5 mm[9]. For flow visualization, transvaginal color Doppler was used. All patients rested in a waiting room for at least 20 min before being scanned, in order to minimize any possible effects of exercise on uterine and ovarian blood flow. The examination was performed with the patients in the lithotomy position, using color Doppler sonography with a 5-MHz transvaginal probe for imaging and 6-MHz pulsed Doppler system for blood flow analysis (Hitachi-Medico EUB-515 A, Hitachi, Tokyo, Japan). All scans were performed by one operator (A.N.).

For each examination, the probe was covered with a coupling gel, inserted into a condom, and recoated with gel before insertion into the vagina. Color flow images of the uterine arteries were sampled lateral to the cervix in a longitudinal scanning plane in order to accurately identify a consistent anatomical position. The probe was first directed into the right vaginal fornix and the ascending branch of the right uterine artery was located along the lateral border of the uterus. The Doppler gate was then placed over the investigated vessel. Angle corrected velocity waveforms were obtained for each uterus with isonation angles of less than 30°. The sample volume of the Doppler system was set at 3 mm and a 100 Hz high pass filter was used to reduce the noise from the pulsating arterial wall. Pulsed Doppler waveforms were displayed at sweep speeds of 40～80 mm/sec. The time-averaged mean velocity was calculated with machine software (Hitachi-Medico EUB-515 A, Hitachi, Tokyo, Japan) by placing electronic calipers on Doppler tracings displayed on the image monitor. At least three similar, sequential Doppler waveforms were recorded and the single best tracing obtained in each artery was selected for analysis. On the duplex image, which showed both the longitudinal two-dimensional image of the uterine artery and the Doppler waveform, the crosssectional area of the artery was measured at the same point by mapping the perimeter of the vessel lumen with a "tracer ball" at right angles to the vessel. The left uterine artery was located and studied in an identical manner.

Uterine arterial blood flows were calculated from the product of the measured time-averaged velocity of blood in the vessel and the cross-sectional area of the lumen of the vessel.

All data were expressed as mean ± standard

Table 1 Characteristics of subjects

	Spontaneous cycles (n = 60)	Stimulated cycles (n = 37)
Age (yr) *	32.2 ± 4.1	33.0 ± 5.1
Nulliparity #	53 (88)	32 (86)
Infertility duration (yr) *	3.4 ± 2.2	3.6 ± 2.8
Menstrual cycles (day) *	29.5 ± 1.3	30.2 ± 1.2
Maximum follicular diameter (mm) *	19.8 ± 1.3	20.5 ± 2.0
Maximum endometrial thickness (mm) *		
Follicular phase	11.3 ± 1.7	10.9 ± 1.5
Luteal phase	12.8 ± 2.1	12.6 ± 1.9

* Data are given the mean ± SD.
# Data are the number of patients. Number in parentheses are the percentage.
Not significant.

deviation (SD). One-way analysis of variance followed by Scheffés *F* test was used to compare the values within each study group. Mann-Whitney *U* test was also used to compare the values between the spontaneous and the hormonally stimulated cycles. Differences with a *P* value of less than 0.05 were considered to be statistically significant.

Results

All 97 subjects completed the study. The characteristics of the subjects are reported in **Table 1**. There were no significant differences in these parameters between the spontaneous and the hormonally stimulated cycles.

The Doppler waveforms were analyzed from 12 days before ovulation to the 12th day after ovulation. In all subjects, there was no significant difference between the right and left uterine arteries for the mean and range of blood flow values. The mean and standard deviation for uterine arterial blood flows in the two study groups are shown in **Fig. 1**.

In the spontaneous menstrual cycles, the uterine arterial blood flow increased significantly from the follicular phase to the day of ovulation and then increased markedly to about 200～230% of the follicular phase after the 6th day of ovulation. In contrast, the uterine arterial blood flow in the clomiphene citrate stimulated cycles did not change at the day of ovulation and then increased significantly to about 180～220% of the follicular phase after the 6 th day of ovulation.

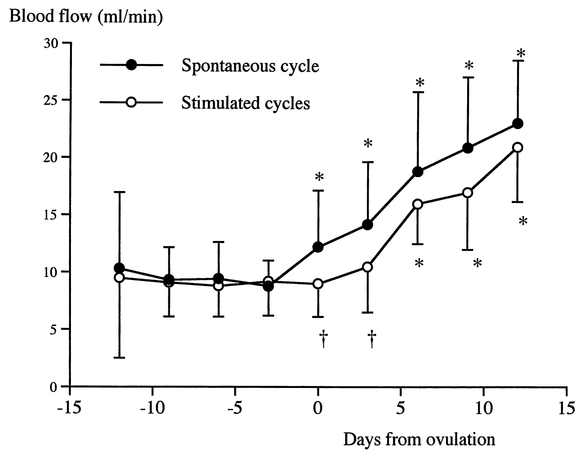

Fig. 1 Changes in blood flow of the uterine artery from the follicular to the luteal phase of the menstrual cycle. Values are presented as the mean ± SD. The closed and open circles indicate the spontaneous and stimulated cycles, respectively. *Significant difference was found between the follicular and the luteal phase in each study group (one-way analysis of variance followed by Scheffe's *F* test, p< .05). †Significant difference between the spontaneous and stimulated cycles (Mann-Whitney *U* test, p < .05).

Discussion

In the present study, we demonstrated that endometrial perfusion was lower in stimulated menstrual cycles than in spontaneous menstrual cycles during the periovulatory period.

Because ultrasound is noninvasive, easily repeatable, and specific, the use of this tool as a replacement for the histological evaluation of an endometrial biopsy specimen was initially assessed in endometrial dating. Endometrial thickness is easy to measure by echography. The first question to be posed is whether endometrial thickness is greater in conception cycles. Friedler et al.,[10] in their review of 22 studies that included 4,256 cycles, have analyzed the role of ultrasonography in the evaluation of uterine receptivity. In 16 of the 22 studies that Friedler examined, there was no significant difference between the conception and nonconception cycles. In agreement with those findings, our results on the mean endometrial thickness were virtually the same in spontaneous and stimulated menstrual cycles during the whole period of the follicular and luteal phase. The criticisms that apply to endometrial thickness have prompted the recent shift of attention from analysis of the anatomical dimension to the physiological dimension.

Several studies show that the measurement of uterine arterial blood flow by Doppler ultrasound is a good method of assessing uterine receptivity during the treatment of infertility[4,6,11,12]. It is apparent that there is a relationship between the concentration of ovarian hormones in peripheral venous plasma and uterine artery blood flow parameters[3,4,6,13–15]. Studies on flow waveforms of the uterine artery during the normal menstrual cycle show a sharp increase in end-diastolic velocities between the proliferative and secretory phases of the menstrual cycle[15,16]. It is particularly interesting that the lowest flow impedance is recognized during the time of peak luteal functioning[4,13,15,17], during which implantation is most likely to occur. Contrary to this, in anovulatory cycles these changes do not occur, and a continuous increase in vascular resistance is seen[15]. Our results on the endometrial perfusion in both the spontaneous and stimulated menstrual cycles after the 6 th day of ovulation are in good agreement with those in the previous studies, but during the periovulatory period there were the conflictive results. Between the day of ovulation and the 3 rd day after ovulation, an increase in endometrial perfusion was observed in the spontaneous menstrual cycles, but not in the stimulated cycles.

Subsequent authors[18,19] who examined the predictive value of transvaginal uterine Doppler assessment in an in-vitro fertilization (IVF) program have observed no difference between pregnant and nonpregnant cases. In addition, our preliminary data[7] also demonstrated that although lower vascular impedance of the uterus occurred during the day of ovulation in the conception cycles, during the luteal phase there were no differences in the vascular impedance of the uterus between the conception and nonconception cycles. A decrease in the impedance of the uterine artery occurring in the conception cycles in the preliminary study was evidence of the increase in blood supply during the day of ovulation. This makes it likely that the blood flow in the ovulatory phase is a fundamental prerequisite for the structural changes and increased vascularization in the endometrium prior to successful conception.

Based on these results, we suggest that lower endometrial perfusion during the periovulatory period in stimulated menstrual cycles in this study may be an important contributing factor to some cases of infertility and that women with poor uterine perfusion during the periovulatory period should be advised that pregnancy is unlikely in their current treatment cycle.

One possible explanation for the lower uterine perfusion seen in the induced ovarian cycles is that clomiphene citrate is shown to deplete estrogen receptors in estrogen-sensitive tissues influencing both endometrial growth and pattern[20]. Goswamy et al.[13] show that administration of estradiol can improve uterine response in cases of poor uterine perfusion. The present results also suggest the presence of better uterine receptivity during the periovulatory period of spontaneous ovarian cycles. Although these findings suggest that this drug may cause a reduction in uterine arterial blood flow, the mechanisms responsible for this effect are not completely clear. There is an urgent need for further studies to clarify these problems.

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