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Hemodynamic response of ovarian artery after hCG injection

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Abstract

We have analyzed ovarian hemodynamics immediately after human chorionic gonadotropin (hCG) administration in patients treated by clomiphene-hCG and human menopausal gonadotropin-hCG. This study involved 40 infertile women who signed consents to participate in this study. After intramuscular injection of 10 000 IU hCG, the change of ovarian arterial blood flow (BF) was evaluated by color Doppler. Pulsatility index, resistance index, maximum velocity (V_{max}), mean velocity, minimum velocity, cross-sectional area of ovarian artery (Area) and BF were measured before and 15–180 min after hCG administration. In the 36 subjects in which ovulation was induced successfully, V_{max} and BF increased significantly even at 15 min after hCG administration and thereafter. In the 4 non-ovulatory subjects, no significant changes in any of indices at any of measured time points were observed. Comparative study of non-ovulatory and ovulatory subjects suggested that ovulation may be predicted by the ovarian hemodynamic analysis immediately after hCG administration.

Keywords: Ovulation; Ovarian artery; Hemodynamics; Color Doppler; Maximum velocity; Blood flow

1. Introduction

From 1970 to the mid-1980s, many animal studies on ovarian hemodynamics during the ovulatory phase were carried out with sheep (Brown and Mattner, 1984), rats (Piacsek and Huth, 1971; Varga et al., 1985), dogs (Varga et al., 1973) and rabbits (Janson, 1975; Makinoda, 1980). These studies clarified that the ovarian blood flow (BF) was greatly increased immediately after luteinizing hormone (LH) or human chorionic gonadotropin (hCG) administration. The findings of animal studies are likely to occur in human as well; however, studies on human ovarian BF during the ovulatory phase were rarely reported before 1990 because of measuring difficulties. With recent widespread use of pulsed Doppler and color Doppler methods, studies on human ovarian BF have been reported (Kurjak et al., 1991; Schurz et al., 1993; Zaidi et al., 1996; Tan et al., 1996; Lunenfeld et al., 1996) which refer to ovarian hemodynamics during the normal menstrual cycle, but never refer to the drastically changed ovarian BF immediately after LH or hCG administration observed in animal experiments. Therefore, we focused on this increased BF immediately after hCG administration during the pre-ovulatory period, and sought to determine whether or not the same event occurs in humans, using the color Doppler method. Moreover, the possibility of predicting ovulation after hCG administration using various hemodynamic indices was examined by comparing ovarian hemodynamics between ovulatory and non-ovulatory subjects. Finally, the best index for predicting ovulation by hemodynamic analysis was investigated.

2. Subjects and methods

2.1. Subjects

This study involved 40 outpatient women (age: 28.8 ± 0.5, mean ± S.E.M.) who had never undergone gynecological operation, and were diagnosed with ovulatory disorders and treated at the Department of Obstetrics and Gynecology, Kanazawa Medical University Hospital from March 1998 to April 2002. Prior to the study,

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patients were given sufficient explanation, and only patients who signed consents to participate in the study were enrolled as subjects.

2.2. Ovulation induction

2.2.1. Clomiphene citrate/hCG treatment (21 subjects)

Clomiphene citrate (Clomid, Shionogi & Co. Ltd, Osaka, Japan) at doses of 50–150 mg/day was administered continuously for 5 days starting from the 5th day of the menstrual period. When the follicular diameter exceeded 18 mm, 10 000 IU hCG (HCG Mochida, Mochida Pharmaceutical Co. Ltd, Tokyo, Japan) was administered by intramuscular (IM) injection.

2.2.2. Human menopausal gonadotropin/hCG treatment (19 subjects)

Starting between the 3rd and 5th day of the menstrual period, 75–300 IU/day of human menopausal gonadotropin (Humegon, Nippon Organon K.K., Osaka, Japan) was administered by IM. It was continuously administered until the follicular diameter reached 18 mm. As mentioned above, when the follicle reached 18 mm or greater, 10 000 IU hCG (HCG Mochida, Mochida Pharmaceutical Co. Ltd, Tokyo, Japan) was administered by IM.

2.3. BF measurement

2.3.1. Ultrasonic apparatus

A Toshiba SSA-380A color Doppler ultrasonic machine and PVK-651 transvaginal probe (5.0 MHz) were used. Each measurement takes approximately 1 min. Ultrasound exposure (10 mW/cm^2) was equivalent to the level normally used in obstetrical diagnosis.

2.3.2. Measured point of ovarian artery

The subject was placed in the dorsosacral position and an ultrasonic probe was inserted into the vagina as shown in Fig. 1A. The BF of the ovarian artery at the hilum of ovary with dominant follicle was measured by transvaginal ultrasound probe (Fig. 1B).

2.3.3. Measured indices

The following indices were measured each time.- Pulsatility index ($PI = V_{max} - V_{min}/V_{mean}$), resistance index ($RI = V_{max} - V_{min}/V_{max}$). The maximum systolic flow velocity (V_{max}, cm/s). The mean velocity analyzed by tracing (V_{mean}, cm/s). The minimum diastolic flow velocity (V_{min}, cm/s). The cross-sectional area of ovarian artery (Area, mm^2). Blood flow (BF, Area $\times$ V_{mean} $\times$ 60/100, ml/min)

2.3.4. Measured time

Ovarian BF was measured before and at 15, 30, 60, 120 and 180 min after hCG administration.

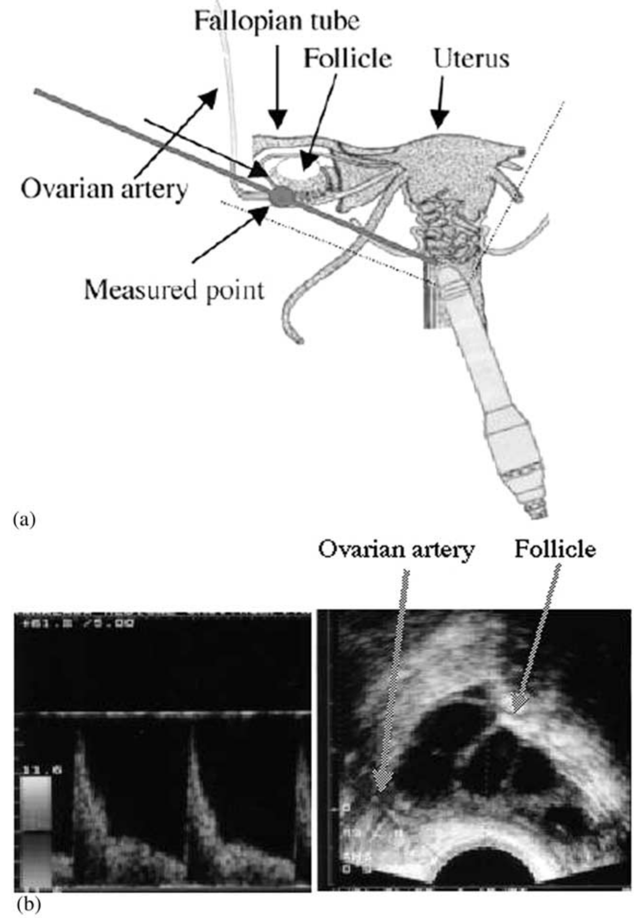

Fig. 1. (a) Measurement area of ovarian artery (Schema). (b) Color flow Doppler image (figure on the right), BF waveform at the sample area (Spectrum) (figure on the left).

2.4. Confirmation of ovulation

Ovulation was confirmed by the disappearance of the follicle at 48 h after hCG administration.

2.5. Assessment of measurement error

Possibilities exist of systemic error in data obtained by ultrasonic diagnosis, such as interobserver and intraobserver error. An intraclass correlation coefficient (R_i) (Oppo et al., 1998) was used to assess interobserver error. The following criteria for clinically relevant agreement were used: poor, R_i less than 0.40; fair, R_i greater than or equal to 0.40 but less than 0.60; good, R_i greater than or equal to 0.60 but less than 0.75; and excellent, R_i greater than or equal to 0.75.

2.6. Statistical analysis

Each measured value is expressed as mean $\pm$ S.E.M. Fisher's PLSD was used for statistical analysis with P values of less than 0.05 being regarded as significant.

3. Results

Of 40 subjects, ovulation was confirmed in 36 subjects (age: 29.0 ± 0.7) and the other 4 subjects (age: 27.0 ± 2.6) showed no ovulation even at 48 h after hCG administration.

3.1. Ovarian hemodynamics in ovulatory subjects

3.1.1. PI and RI

PI before, at 15 and 180 min after hCG administration was 3.4 ± 0.2, 3.3 ± 0.2 and 3.2 ± 0.2, respectively. No significant increase in PI was observed at any time after hCG administration. RI before, 15 and 180 min after hCG administration was 0.92 ± 0.01, 0.92 ± 0.01 and 0.91 ± 0.01, respectively. Similar to PI, no significant change in RI was observed at anytime (Fig. 2).

3.1.2. BF velocity

V_{max} of ovarian BF before hCG administration was 35.2 ± 2.1, which increased significantly to 42.6 ± 2.6 at 15 min after hCG administration. Significant increases in V_{max} were observed thereafter (Fig. 3).

V_{mean} and V_{min} also showed an increase after hCG administration, although it was not as remarkable as V_{max}.

3.1.3. Ovarian arterial BF

Ovarian arterial BF is calculated by multiplying the aforementioned V_{mean} by Area. Area before hCG administration was 6.4 ± 0.7, which increased significantly to 8.9 ± 0.8 at 15 min after hCG administration and continued to increase. A significant increase in Area was observed after hCG administration. Since both Area and V_{mean} increased after hCG administration, a BF of 42.4 ± 5.2 before hCG administration significantly increased to 66.8 ± 6.7 at 15 min after hCG administration. Significant increases in BF were observed after hCG administration (Fig. 4).

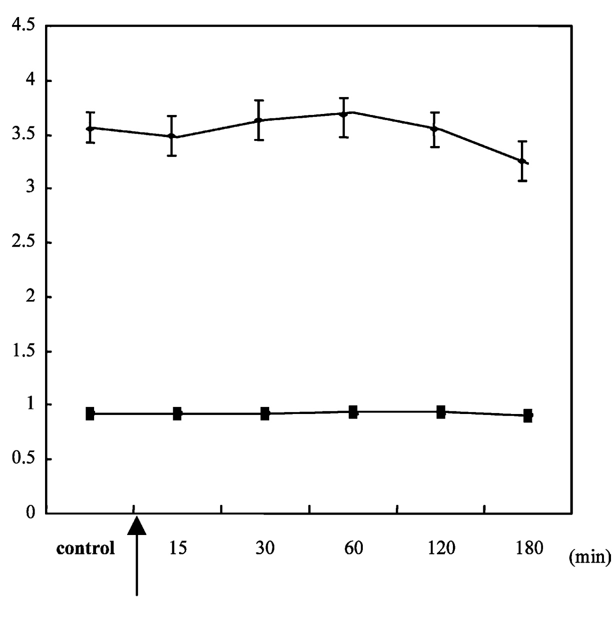

Fig. 2. Changes in PI and RI after hCG administration in ovulatory subjects; PI, ($PI = V_{max} - V_{min}/V_{mean}$); RI, (RI, $V_{max} - V_{min}/V_{max}$; (◆) PI; (■) RI (mean ± S.E.M.).

3.2. Ovarian hemodaynamics in non-ovulatory subjects

3.2.1. Maximum systolic flow velocity (V_{max}) and ovarian arterial BF.

No significant changes in any indices including V_{max} and BF were observed at anytime after hCG administration (15, 30, 60, 120 and 180 min) as compared to before administration (Fig. 5).

3.3. Intraclass correlation coefficient

The coefficient of PI and RI is 0.928 and 0.899, respectively, showing excellent correlation. Both PI and RI are low-error indices. The coefficient of V_{max} was 0.837 showing also excellent correlation. The coefficient of BF was 0.615 showing good correlation. Based on these results, V_{max} was a less error-prone index as compared to BF (Table 1).

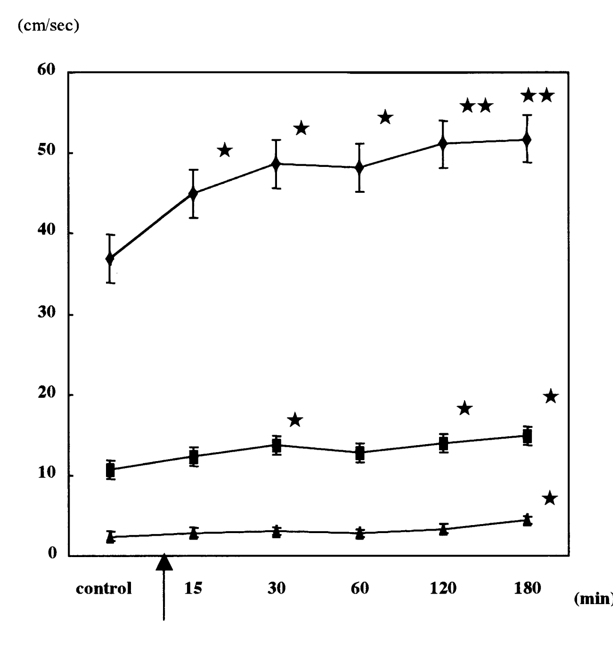

Fig. 3. Changes in V_{max}, V_{mean} and V_{min} after hCG administration in ovulatory subjects; V_{max} (the maximum systolic flow velocity), V_{mean} (the mean velocity analyzed by tracing), V_{min} (the minimum diastolic flow velocity). (◆) V_{max}; (■) V_{mean}; (▲) V_{min} (mean ± S.E.M.). Significant difference compared to before hCG administration ($*P < 0.05$, $**P < 0.001$).

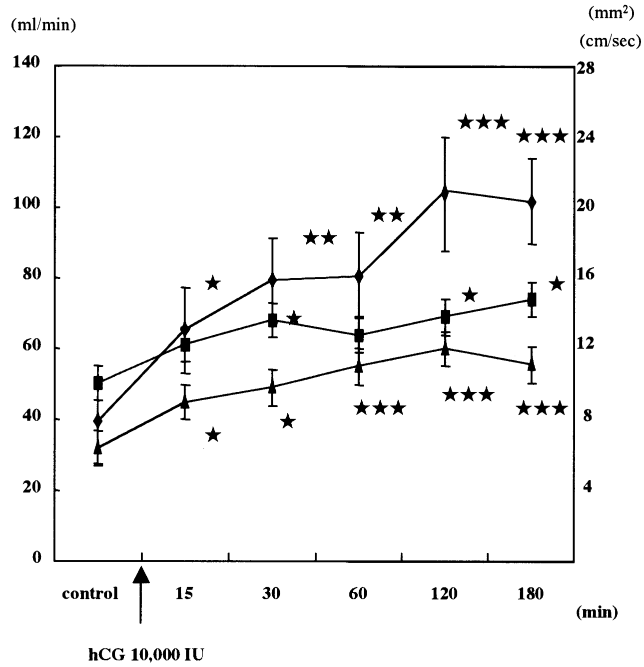

Fig. 4. Changes in BF, V_{mean} and area after hCG administration in ovulatory subjects; BF (the cross-section of ovarian artery × V_{mean} × 60/100), V_{mean} (the mean velocity analyzed by tracing), Area (the cross-section of ovarian artery). (◆) BF; (■) V_{mean}; (▲) Area (mean ± S.E.M.). Significant difference compared to before hCG administration (* $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$).

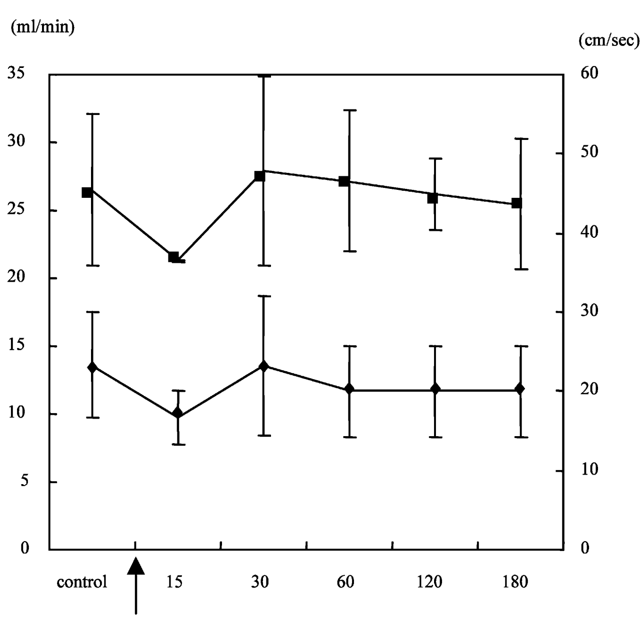

Fig. 5. Changes in V_{max} and BF after hCG administration in non-ovulatory subjects; V_{max} (the maximum systolic flow velocity), BF (the cross-section of ovarian artery × V_{mean} × 60/100), (◆) BF; (■) V_{mean} (mean ± S.E.M).

4. Discussion

It has long been expected that ovarian hemodynamics are greatly changed around the period of ovulation. In 1971, Piacsek and Huth used a drop-flow counter method and measured the amount of ovarian BF through the ovarian vein. They reported that rat ovarian BF was increased immediately after LH administration. In the microsphesre study by Janson (1975), rabbit ovarian BF was rapidly increased by approximately 30 and 75% at 2 and 20 min after LH administration, respectively. In 1980, Makinoda inserted a crossed-thermocouple into rabbit ovaries and measured the ovarian BF after hCG administration. He reported that ovarian BF was rapidly increased by approximately 20, 30 and 50% at 15 min, 1 and 2 h, respectively, and remained at high levels for 3–5 h, gradually decreasing thereafter towards ovulation.

Since rapidly increased ovarian BF immediately after LH or hCG administration was a common event in the animal studies, this event is also likely to occur even in human. However, drop-flow counter, microspheres and needle-type crossed-thermocouple methods are invasive methods that cannot be applied in clinical studies. Due to methodological difficulties, there are no reports on increased ovarian BF immediately after hCG administration to the best of our knowledge.

Great strides have been made in the field of BF measurement with recent developments in medical engineering. Ultrasonographic diagnoses such as pulse Doppler, color Doppler and power Doppler are non-invasive methods that can be used clinically. In this study, changes in the human ovarian BF were measured by color Doppler, which is commonly used in the fields of obstetrics and gynecology. Color Doppler when used for outpatient services enables measurements to be done in a relatively short time. Since operation is non-technical, examiners can use it by themselves and immediately present the results to patients. Ultrasonographic diagnosis including color Doppler is a very

Table 1
Intraclass correlation coefficient

Poor:	$R_i < 0.40$	Fair:	$0.40 \leqq R_i < 0.60$
Good:	$0.60 \leqq R_i < 0.75$	Excellent:	$0.75 \leqq R_i$
PI	0.928	Excellent	
RI	0.899	Excellent	
V_{max}	0.837	Excellent	
V_{min}	0.875	Excellent	
V_{mean}	0.717	Good	
Area	0.623	Good	
Blood flow	0.615	Good	

Intraclass correlation coefficient ($\sigma_b^2 - \sigma_w^2/\sigma^2$; external variance,$\sigma_b^2$; internal variance, σ_w^2; total variance, σ^2).

common method of general treatment, and subjects are familiar with it.

In the field of obstetrics, only PI and RI are commonly used as indices for measurement of BF and these indices have sufficient clinical value, since they present changes in waveform. In other words, PI and RI cannot be used, if no remarkable changes are observed in waveform. The purpose of this study is to confirm changes in BF a short time after hCG administration which have been already found in animal experiments. In such short time, we assumed that the changes of waveform could not be observed and the same part on the ovarian artery could be easily assessed. For these reasons, we have adopted the indices such as V_{max} and BF. Brännström et al. (1998) reported that the maximum BF velocity at the apex of the follicle decreased and the maximum BF velocity at the base of the follicle increased at the time of ovulation. Since study by Brännström et al. was successfully performed on the vessels around the follicle, our clinical trial on ovarian artery, whose diameter is far greater than the follicle's blood vessel, could also be carried out successfully.

This study resulted in a significant increase in ovarian arterial BF being observed in all indices except PI and RI in ovulated subjects after hCG injection. No significant changes in PI and RI were attributed to the fact that waveform during each heartbeat did not show any change. Significant increases in V_{max} and BF at 15 min after hCG IM injection is a worthwhile finding, since this suggests that the results of many animal experiments have been confirmed to occur also in human.

Four non-ovulatory subjects had no significant changes in any indices after hCG injection. Although there are various factors responsible for non-ovulation, a lack of increase of ovarian arterial BF may be used as a predictor of non-ovulation. This study was the first to clarify that ovarian BF was increased immediately after hCG injection in the normal ovulation process in human.

This finding provides examiners legitimate reasons to suspect that disorders may occur somewhere during the ovulation process if ovarian BF is not increased. A comparative study of non-ovulatory and ovulated subjects suggested that ovulation may be predicted by the ovarian hemodynamic analysis immediately after hCG administration. Results of BF measurement are slightly more accurate than those of V_{max}. However, the vascular cross-sectional area necessary to calculate BF is difficult to measure. Recent color Doppler tends to show images larger than the actual vascular cross-section.

In this study, the coefficient of V_{max} was 0.837, showing excellent correlation. The coefficient of BF was 0.615, showing good correlation. Based on these results, V_{max} was shown to be a less error-prone index as compared to BF. If the vascular cross-section is accurately measured with the development of ultrasonic diagnosis in the future, BF should be an efficient index. Until then, V_{max} with less error might be the most efficient index.

In conclusion, we have measured the ovarian BF using V_{max} and other indices. V_{max} in ovulated cases showed the immediate increase after hCG administration. In contrast, no significant changes were observed in non-ovulated cases. These results indicate that the ovulation might be predicted by the color Doppler analysis after hCG administration. However, since the number of unovulated cases is too small, further study is necessary.

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