Doppler velocimetry of the ovarian artery as a tool to detect LH surge in stimulated cycles

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Abstract

Our aim was to assess the velocimetric pattern of the ovarian artery as a possible marker of LH surge in stimulated cycles. A total of 130 women undergoing ovarian stimulation for intrauterine insemination were randomized in two groups. Each woman was stimulated with 75 IU of recombinant FSH starting from the third day of the cycle. Velocimetric indices of the dominant ovarian artery were compared between patients with spontaneous LH surge and those needing HCG administration to trigger dominant follicle rupture. The pulsatility index and the ratio between peak systolic flow and lowest diastolic flow were significantly higher in women that had a spontaneous triggering of ovulation. These parameters had a high and very significant positive correlation with the dosage of luteinizing hormone. Threshold values of 2.60 for PI and 7.68 for S/D had a high sensitivity and specificity to predict LH surge. These velocimetric results demonstrated that an increased resistance in the dominant ovarian artery is correlated to LH surge in stimulated cycles. It may represent a sign of relevant clinical utility in timing of intrauterine insemination and/or natural intercourse.

Introduction

Ovulation is a complex physiological mechanism whereby an egg ready to be fertilized is released from the ovary. The identification of the ovulation time is a key factor of the follicular monitoring in spontaneous and stimulated cycles so much so that several techniques have been proposed to predict it [1,2]. Most of them are indirect methods monitoring the basal body temperature and cervical mucus checking. The only direct method is the observation of follicle rupture by laparoscopy, but it cannot be performed on a routine basis. Therefore, most frequently used methods are the luteinizing hormone (LH) peak and detection of some ultrasound signs. Several studies described the ultrasound indices of ovulation. However, their sensitivity and specificity are too low [3]. Vascular changes within the dominant ovary have been investigated throughout the normal menstrual cycle by means of 3D Doppler ultrasound. This method shows that, along with the formation of capillaries invading the corpus luteum granulosa cell, a rise of ultrasound indices occurs, reflecting the increase of both density of blood vessels and number of blood corpuscles flowing in the area [4]. However, no correlations were found between such vascular indices and both LH and progesterone levels, making them unsuitable to the purpose of identifying the time of ovulation. These physiological changes of the ovarian vascularization have been confirmed in others studies [5] stimulating further research in this field; in particular, the present study aimed to assess the Doppler velocimetric pattern of the ovarian artery as a new possible marker of LH surge.

Materials and methods

We conducted a prospective randomized study enrolling 130 consecutive patients – admitted to the Fertility Center of Ferrara University from June 2011 to June 2012 – to perform controlled ovarian stimulation (COS) for intrauterine insemination (IUI). Inclusion criteria were: unexplained sterility, age between 25 and 40 years, presence of a single dominant follicle, follicle size between 18 and 22 mm, regular menstrual cycle (26–30 days). Exclusion criteria were: abnormal cycles (polycystic ovary disease or luteal defect), FSH >15 mIU/mL, frequent anovulatory cycles, symptoms and clinical signs related to endometriosis, vascular disease such as hypertension and chronic venous insufficiency. A complete medical history was obtained and clinical examination was made on both partners. Couples were investigated with ovarian reserve test, semen analysis and the tubal patency test. The patients were stimulated according to the protocol of our Fertility Center. The ovarian stimulation with 75 IU of recombinant FSH started on the third day of the menstrual cycle. The first examination was made on the 8th day and included ultrasound follicular monitoring and serum samples for estradiol (E2), progesterone (PGR) and LH levels measured using radioimmunoassay. The time of the following examination was decided according to the result of the monitoring. Written informed consent was obtained from all study subjects. Our study was approved by the Local Ethic Committee. Before entering the treatment cycle, the patients were randomly divided into two
groups according to a computer-generated list by using opaque sealed envelopes. Group A (expectant management) included 65 patients in whom a spontaneous triggering of ovulation, by detecting LH raise with serial serum testing, was awaited (the spontaneous LH surge was defined as an increase in the LH levels >100% over the mean of the preceding 2 days). Group B (control group) included 65 patients in whom a mature follicle was displayed but without biochemical signs of triggering ovulation (follicular maturation parameters were: size ≥18 mm, E2 >300 pg/ml, no LH surge).

Ultrasound follicular monitoring was performed using a 7 MHz vaginal probe (ALOKA PROSOUND ECOCGRAF SSD3500SX). All examinations were performed between 8.00 h and 9.00 h, with the patient in the lithotomy position. Dominant follicle was identified and measured through the mean of the two larger diameters. The ovary site of the dominant follicle was defined as the ‘dominant ovary’. Arterial flow signals of the ovarian branch of uterine artery were obtained using color Doppler in the two-dimensional mode. A 2-mm range gate was then placed across the vessel, ensuring that the angle between the Doppler beam and the vessel length was close to 0 degrees. A 50–100 Hz filter was used to eliminate low-frequency signals due to the movements of the vessel wall. Pulsatility index (PI), resistance index (RI) and peak systolic flow velocity (PSV) were calculated electronically when three similar, consecutive waveforms of good quality were obtained. The parameters were analyzed through software for calculating the ratio between peak systolic flow and lowest diastolic flow (S/D) and were compared at two different times.

Time 0 (T0) represented the beginning of the stimulation cycle (day 3) while Time 1 (T1) represented the spontaneous triggering of ovulation for group A (LH levels >100% over the mean of the preceding 2 days) and HCG administration for group B (mature follicle observed on ultrasound without biochemical signs of triggering ovulation). All scans were performed by the same operator (E.G.) to avoid interobserver variation.

All data obtained were recorded and analyzed using SPSS (19.0 for MAC) program. t Test was used for continuous variables and chi-square for categorical variables. Pearson test was used for assessing the correlations between velocimetric indices and the hormonal variables. The receiver operating characteristic (ROC) curve was used to analyze the specificity and sensitivity, and the area under the ROC curve was used to represent an overall summary of diagnostic accuracy. A p value <0.05 was considered statistically significant. A confidence interval (CI) of 95% was applied where appropriate.

**Results**

Twenty-two patients (15 of the group A and 7 of the group B) were excluded from the study for various reasons (multifollicular development, personal reasons, follicle size >22 mm, premature LH surge). The flow of the patients through study is shown in Figure 1. Therefore, the study population was composed by 108 patients. Table 1 shows the baseline characteristics of the two groups. In particular, the hormonal characteristics such as the level of FSH, E2, LH on the third day of the cycle and of PGR in luteal phase are displayed. For each assessed variable, significant differences between the groups are outlined as well. Hormonal characteristics were also compared between the two groups at T0 and T1 showing a significant different value of E2 and PGR at both considered times (Table 2). Comparison of the velocimetric indices of the dominant ovary between the two groups showed a highly significant difference in PI and S/D values (Table 3). According to the results of the Pearson correlation, assessing the correlation of the velocimetric indices in the dominant ovary with the biochemical markers of ovulation such as PGR and LH at T1 in the group A, we found:

- High, very significant positive correlations between PI and S/D with LH at T1 (r = 0.631, p = 0.0005; r = 0.723, p = 0.0005 respectively);
- Low, very significant positive correlation between RI and LH at T1 (r = 0.38, p = 0.01);
- Low, very significant positive correlation between PI, RI and S/D with PGR at T1 (r = 0.423, p = 0.0005; r = 0.270, p = 0.005; r = 0.323, p = 0.004 respectively).

**Table 1.** Baseline characteristics of the patients between the two groups.

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 50)</th>
<th>Group B (n = 58)</th>
<th>CI 95%</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>37.00 (±4.3)</td>
<td>35.29 (±3.3)</td>
<td>0.23 to 3.17</td>
<td>0.02*</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>6.54 (±1.3)</td>
<td>7.90 (3.2)</td>
<td>−2.43 to −0.30</td>
<td>0.01*</td>
</tr>
<tr>
<td>E2 (pg/mL)</td>
<td>86.40 (±53.67)</td>
<td>67.05 (±45.5)</td>
<td>0.09 to 38.58</td>
<td>0.04*</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td>6.32 (±2.5)</td>
<td>6.00 (±2.3)</td>
<td>−0.65 to 1.28</td>
<td>NS*</td>
</tr>
<tr>
<td>PGR (ng/mL)</td>
<td>12.97 (±6.5)</td>
<td>12.11 (±9.3)</td>
<td>−3.00 to 4.70</td>
<td>NS*</td>
</tr>
<tr>
<td>TSH (mIU/L)</td>
<td>1.83 (±0.9)</td>
<td>2.22 (±1.1)</td>
<td>−0.83 to 0.06</td>
<td>NS*</td>
</tr>
<tr>
<td>PRL (ng/mL)</td>
<td>25.15 (±9.6)</td>
<td>22.56 (±13.5)</td>
<td>−2.48 to 7.66</td>
<td>NS*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.30 (±2.9)</td>
<td>23.76 (±3.5)</td>
<td>−1.76 to 0.83</td>
<td>NS*</td>
</tr>
<tr>
<td>Duration infertility (y)</td>
<td>2.25 (±0.8)</td>
<td>2.85 (±0.9)</td>
<td>−0.96 to −0.24</td>
<td>0.001*</td>
</tr>
<tr>
<td>Type of infertility</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>36</td>
<td>52</td>
<td></td>
<td>NS#</td>
</tr>
<tr>
<td>Secondary</td>
<td>4</td>
<td>16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FSH, E2, LH levels were measured on cycle day 3. PGR level was measured on cycle day 21.
Statistical method: *t test; #chi-square.
The ROC curve showed that the best parameters in predicting the ovulation were PI, RI and S/D (the areas under the curve are 0.852, 0.801 and 0.801, respectively; Figure 2). The best threshold values were 2.60 (sensitivity 75%, specificity 100%, LR 3.4) for PI, 7.68 (sensitivity 75%, specificity 82%, LR 4.1) for S/D and 0.87 (sensitivity 75%, specificity 82%, LR 1.7) for RI.

Conclusions

Ovulation is not only the simple rupture of the follicle, but also includes a sequence of physiologic mechanisms that induce oocyte maturation and major phenotypic changes in the mural granulosa and cumulus cells culminating with release of the egg [6]. The key factor responsible for this process is represented by the surge of LH, because it terminates the program of FSH-dependent steroidogenesis and granulosa cells growth, while promoting differentiation of somatic cells into luteal cells. At the same time, LH induces the expression of genes required for follicle rupture [7]. Therefore, the detection of ovulation is fundamental for planning the best timing for natural intercourse and IUI [8]. Consequently, a plethora of techniques have been suggested to predict ovulation in the last thirty years [3]. The first tested methods were the monitoring of the basal body temperature and cervical mucus checking, however their sensitivity was too low [9]. Instead, plasmatic rise of LH represents the best predictor of ovulation [10] but it is rather expensive and invasive. Therefore, through the years a variety of urinary hormone-based methods have been suggested but with different results [11]. In addition, ultrasound plays an important role, based on the following features: disappearance or sudden decrease in follicular size, increased echogenicity and irregularity of follicular wall, and appearance of free fluid in the pouch of Douglas [3]. Disappearance or sudden decrease in follicular size is the most frequent sign of ovulation but reported sensitivity varies too widely (from 11% to 84%) [12]. Increased echogenicity of the follicle content is a doubtful sign. Indeed, besides indicating a transition from follicle to corpus luteum, it may also derive from the pre-ovulatory cumulus oophorus. Accordingly, its reported sensitivity is low (about 35 %) compared to irregularity of follicular wall and free fluid in the pouch of Douglas (61% and 71%, respectively) [12]. However, equipment and software ultrasound have improved significantly over the years and methods such as Color Doppler allow us to study the vascularization of small vessels [13]. Consequently, research focused on the study of utero-ovarian vascular district thus obtain significant positive results, especially in Obstetrics [14]. Therefore, considering the vascular changes in the ovary site of dominant follicle during ovulation [4,5], the Color Doppler study of the ovarian artery might represent an interesting field of research. For these reasons, our study aimed to verify significant velocimetric changes in dominant ovarian artery during the triggering ovulation in order to identify a possible new predictive marker.

We chose the plasmatic peak of LH being one of the more reliable test indeed, several authors demonstrated that IUI performed 18–24 h after the onset of the LH surge has better success rate [15,16]. Therefore, the identification of an ultrasound marker comparable to the LH surge could simplify the timing of IUI and natural intercourse.

Analyzing our data we can note some differences in the baseline characteristics of the two groups in the Table 1.
Nevertheless, it should be highlighted that the different mean value of FSH and E2 is statistically significant but not clinically relevant because it is in the normal range. In Table 2 other differences are visible, namely the different concentration of E2 and LH between the two groups at T1. Such a result may be explained by different management between the two groups, namely waiting for spontaneous ovulation in group A and identification of a mature follicle without the LH surge in group B. Table 3 shows significant differences among the velocimetric indices measured in the dominant ovarian artery. In particular, the statistical analysis shows a value of PI significantly higher in group A, while RI value is higher in group A but does not meet statistical significance. The reason for a lack of a significant difference between RI values may be found to the particularly narrow range of values. Further evidence for the statistical significance of differences in the velocimetric behavior between the two groups is expressed by S/D analysis which value results higher in group A. Overall, our data indicate an increase of velocimetric indices (PI and S/D) in group A, which indicate an increased vascular resistance in the dominant ovarian artery of the patients with a spontaneous triggering of ovulation. They do not contradict the previous observations that demonstrated a decreased vascular resistance in the intraovarian arteries during the luteal phase [17]. Rather, taken together, indicate that an increased arterial tone during the preovulatory phase heralds a subsequent intra-ovarian resistance decrease aimed to increase the blood perfusion for functional purposes of the corpus luteum [17]. Such a perspective is enforced by the highly significant correlation of both PI and S/D with LH serum level. Indeed, the hormone is known to induce the release of proteases and vasoactive substances [7] that are responsible for the vascular changes identified by Doppler velocimetry. These findings are confirmed by the ROC curve (Figure 2) showing the high predictivity of velocimetric indices (in particular PI) in detecting ovulation.

In the past, several authors studied the utero-ovarian Color Doppler velocimetry. For instance, Chien et al. [18] examined the changes in utero-ovarian blood flow during the peri-implantation period for determining a time interval within the menstrual cycle that was an optimal endometrial status for embryo implantation in assisted conception programs. Other authors [19] investigated a possible marker to predict a fertile cycle by comparing uterine and ovarian blood flow in the follicular phase of the menstrual cycle in women who conceived and women who did not conceive during that cycle. Others [20] studied the possible predictivity of intraovarian artery Doppler indices on pregnancy outcomes. However, there are some considerations whose make our study different. First, the ultrasound method is different because most authors studied intraovarian and stromal arteries. For instance, Adali et al. [21] evaluated blood flow velocity in the ovarian stromal artery in patients with polycystic ovary syndrome while Du et al. [22] examined the correlation between the PI and RI of the intraovarian artery on the day of follicle aspiration and the oocyte quality. Second, the parameters measured are different since our study is the first that, in order to detect a new possible marker of ovulation, compared the Doppler indices of the dominant ovarian artery in women that had a spontaneous triggering of ovulation to those of women that had no signs of ovulation in the same phase of menstrual cycle.

In this perspective, our findings are not comparable with previous ones because no similar evidences are available in the literature. High significance expressed in our data demonstrates no doubt about the phenomenon of increased vascular resistance in the dominant ovary during the preovulatory phase. Our statistical analysis also identified a threshold value for PI with high sensitivity and specificity. Therefore, if these data were confirmed on a larger sample size, the possibility of detecting a new marker of ovulation would become reality. This could improve and simplify the follicular monitoring avoiding unnecessary and expensive blood tests. However, even though these results seem promising, the velocimetric trend should be studied and confirmed in other common clinical conditions such as polycystic ovary disease, multifollicular growth and deficiency of luteal phase. Therefore, the importance of our findings is significant because could greatly stimulate research in this direction.

Declaration of interest

The authors have no conflict of interest to declare.

References


