

## The Fluctuation in the Heart Rate Variability Throughout Ovulation Induction Cycle: Is the Case Different in Polycystic Ovary Syndrome?

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### Abstract

**Objective:** Women with Polycystic Ovary Syndrome (PCOS) have an increased level of sympathetic nerve activity, which was identified by Heart Rate Variability (HRV). PCOS is a state of ovulatory dysfunction in which there may also develop an abnormal ovary response to gonadotropins. We aimed to compare the changes in the HRV of ovulatory women with PCOS and normoovulatory infertile women throughout their ovulation induction cycles.

**Methods:** We included 38 infertile women in the study, 18 of them oligo/amenorrheic with PCOS and 20 eumenorrheic, who formed the control group. Using power spectral analysis, the HRV indices of the patients were examined in three phases of the ovulation induction cycle with gonadotropins.

**Results:** The HRV indices on the menstrual and mid-luteal period were lower in the PCOS group compared to control group. In the PCOS group, the fluctuation was not observed in the HRV indices throughout the ovulation induction cycle. In the control group, the HRV indices were lower on the mid-luteal period and peri-ovulatory period compared to menstrual period.

**Conclusion:** In anovulatory women with PCOS, HRV fluctuation is different from the normoovulatory women throughout the ovulation induction cycles. There might be a link between the disturbed ovulation processes in PCOS and the reduced intra-cycle HRV fluctuation.

**Keywords:** Polycystic ovary syndrome; Heart rate variability; Ovulation induction

### Introduction

Polycystic Ovary Syndrome (PCOS), in addition to being an endocrine and metabolic disorder, is also the most common cause of ovulatory dysfunction in female infertility [1]. Although the etiology of PCOS is not exactly known, some have argued that stress and adrenergic activation might be playing a role in its development [2]. It has been demonstrated in previous studies that there is an increase in the sympathetic nerve activity of women with PCOS [3-6].

It has been argued that sympathetic dominance may have a contributing role in the increase of the prevalence of vascular disease in patients with PCOS [2]. Sympathetic hyperactivity may lead to hypertension as well as to life-threatening ventricular arrhythmias [7].

The biological rhythmicity of the regular menstrual cycle is shaped through the interaction of the hypothalamic, hypophyseal and ovarian hormones. The variations in the functions of the Autonomic Nervous System (ANS) are linked with the hormonal fluctuation in the menstrual cycle [8]. The functions of the ANS in various phases of the menstrual cycle were examined in some studies. Heart Rate Variability (HRV), which is a measure of the cardiac autonomic tonus, displays physiological changes throughout the menstrual cycle [9]. Various studies dealing with HRV have demonstrated that the parasympathetic nerve activity is predominant in the follicular phase, while, because of parasympathetic inhibition, the sympathetic nerve activity becomes predominant in the luteal phase [8,10-12].

In the ovulation induction of women with PCOS, problems like lack of response or exaggerated response were seen more frequently than in the case of normoovulatory women [13]. In the ovulation induction of women with PCOS, carried out with gonadotropins, there is an increase in ovarian hyper stimulation and the risk of multiple pregnancies [14]. In our study, we hypothesized that the ANS fluctuations observed in women with PCOS and sympathetic hyperactivation would be different from those observed in the control group. For this purpose, through

HRV analysis, we compared the ANS changes observed in anovulatory women with PCOS and in normoovulatory infertile women in three phases of their cycles of ovulation induction with the help of gonadotropins.

### Materials and Methods

#### Study design

In this prospective and cross-sectional study, through power spectral analysis, we examined the HRV indices on three points of the ovulation induction cycle, namely on the second or third day of menstruation, on the ovulation day (a day after the human Chorionic Gonadotropin (hCG) application), and finally on the mid luteal day (between six to eight days after ovulation). These days, we also monitored the serum E2 and progesterone levels. We determined the mean outcome measure as the comparison of the HRV indices of infertile women with and without PCOS during their ovulation inductions, and the secondary outcome measure as the relation between the ovarian hormones and the HRV indices. We determined the number of cases via power analysis.

#### Subjects

In this study, we selected the patients from those who applied to the infertility clinic of Baskent University Hospital in Konya. We selected

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**Received** August 10, 2013; **Accepted** September 19, 2013; **Published** September 21, 2013

**Citation:** Uckuyu A, Toprak E, Ciftci O, Ciftci FC (2013) The Fluctuation in the Heart Rate Variability Throughout Ovulation Induction Cycle: Is the Case Different in Polycystic Ovary Syndrome? Gynecol Obstet 3: 172. doi:10.4172/2161-0932.1000172

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twenty-five oligo/anovulatory patients, diagnosed with PCOS according to the Rotterdam criteria [15], to determine their suitability for the research. After exclusion of five patients not fulfilling the criteria and the two refusing participate in the study, we included eighteen infertile women with PCOS in our study. For the control group we selected twenty-five normoovulatory patients with non-PCOS related infertility (unexplained or with a mild male factor) as to their suitability for the study. After exclusion of three patients not fulfilling the criteria and the two refusing participate in the study, we included twenty patients in the control group.

The inclusion criteria were as follows: being between twenty and thirty-four years of age and twenty and twenty-nine BMI, having no endocrine diseases such as DM, thyroid disorder or hyperprolactinemia, having no connective tissue or kidney diseases or hypertension, having no first-degree relatives with a history of coronary artery disease, not using vasoactive medication or not smoking. The exclusion criteria were as follows: being total cholesterol level above 220 mg/dl, HDL Ch level below 30 mg/dl, LDL Ch level above 160 mg/dl, and triglyceride level above 240 mg/dl.

### Ovulation induction protocol

All patients underwent vaginal ultrasound on the second or third day of cycle. Patients whose serum E2 levels were <70 pg/ml, FSH <9 mIU/ml and LH <8 mIU/ml were instituted ovulation induction treatment on the same day. Stimulation with recombinant follicle-stimulating hormone (rec FSH) (Puregon, Organon, Istanbul, Turkey) 50-100 IU/day was started according to low dose step-up protocol and maintained for 8-16 days with adjustment according to response of ovary to the dose. When leading follicle rose to diameter of 18-20 mm, we administered 500 mcg of rhCG (Ovitrelle; Serono, Istanbul, Turkey) subcutaneously. Three days after the triggering of ovulation, micronized progesterone were started vaginally (Progestan; Kocak, Istanbul, Turkey) at the dose of 600 mg/day and administered for fourteen days.

### Heart rate variability

All patients underwent three-channel 24-h Holter ambulatory ECG monitoring by using the DMS 300-8 Holter Recorder (Cardioscan, Suprima Holter System, DMS, USA). Recordings were analyzed by Cardioscan 10, HRV Package system (version 10.2.2.0011a) following the adjustment of RR intervals. Analog data were digitized at 200 Hz and edited by a cardiologist. The validation procedure consisted of beat labeling and tagging of noisy regions. The continuous series of RR intervals (tachogram) was obtained and all 5-min segments with a maximum of five isolated ectopic beats were retained for spectral analysis. Recordings with <18 h of data or <85% of qualified sinus beats were excluded. HRV was assessed in both time and frequency domains. The analyses of HRV were performed according to the recommendation of the European Society of Cardiology Task Force. The time domain parameters of HRV were the mean heart rate, standard deviation of all NN intervals (SDNN), root mean squares of successive differences (RMSSD), the mean of the five-min standard deviation of the NN interval (SDNN index), and then of the adjacent interval divided by the total number of all NN intervals (PNN50).

The power spectrum of HRV was measured using fast Fourier transform analysis in three frequency bands: <0.04 Hz (very low frequency, VLF), 0.04-0.15 Hz (low frequency, LF), and 0.15-0.4 Hz (high frequency, HF). We expressed the power of these components as milliseconds squared.

### Laboratory tests

We collected all the blood specimens following a night of fasting. We performed the hormonal and ultrasonographic examinations on the same day as the HRV analyses. Serum levels of Luteinizing Hormone [LH] and insulin were measured by a microparticle enzyme immunoassay method in an AXSYM autoanalyzer (Abbott Laboratories, IL, USA). Serum levels of fasting glucose, High-Density Lipoprotein [HDL] cholesterol, Low-Density Lipoprotein [LDL] cholesterol, and triglyceride were assessed using original kits, using an Abbott-Aeroset autoanalyzer (Chicago, IL, USA). Serum levels of serum total testosterone and thyroid stimulating hormone, estradiol, and progesterone were assessed by a solid-phase competitive chemiluminescent enzyme immunoassay in an Immulite 2000 autoanalyzer using BIODPC reagents (Bio DPC, Los Angeles, CA, USA). HOMA index was calculated by this formula; (fasting glucose in mg/dL/18)×(fasting insulin in μU/mL)/22.5.

### Ethical considerations

Baskent University Ethical Committee approved the working protocol, and the written consents of the informed patients were received and the research was started.

### Statistical analyses

Data analyses were performed using SPSS software (version 13.0, SPSS Inc, Chicago, IL, USA). The normality of the distribution of continuous variables was evaluated by using the Kolmogorov-Smirnov test with Lilliefors correction. The continuous variables with normal distribution were analyzed by unpaired t-test. The continuous variables with non-normal distribution were analyzed by Mann-Whitney U test. The test of Two-way Analysis of Variance was used for the analysis of the data between the three phases of the cycle, and one of the Two-Related-Sample tests, Wilcoxon Signed Ranks Test was used for the comparison of the means of the two groups, which are dependent. The results were expressed as mean ± Standard Deviation (SD) for continuous data. For all analyses, two-tailed P value <0.05 was considered statistically significant.

### Results

The demographic, hormonal and biochemical characteristics of the women in the PCOS and control groups are shown in Table 1. The BMI, HOMA index, total testosterone and triglyceride levels of the PCOS group were higher than those of the control group. In the control group, the HRV indices were lower on the mid-luteal and ovulation days compared to the menstrual day (Table 2). In the PCOS group, no change was observed in the HRV indices throughout the cycle (Table 2). The HRV indices on the menstrual and mid-luteal days were lower in the PCOS group than they were in the control group (Table 3). When Pearson correlation analysis was performed, no correlation was found between the HRV indices and the serum hormone levels in any of the three phases of the menstrual cycle. Similarly, no correlation was found between the BMI and HOMA index, insulin levels and HRV indices.

### Discussion

As far as we know, this study is the only one in which HRV is used to examine the ANS changes throughout the ovulation induction cycles in anovulatory women with PCOS. In this study, we found that women with PCOS have reduced cardiac autonomic modulation, as demonstrated in previous studies [3-6], and ANS fluctuation was reduced in their stimulated ovulatory cycles.

PCOS is not only a reproductive endocrinopathy, but also

	Patients with PCOS n:18	Controls n:20	P value
Age (yrs)	27.8 ± 5.4	29.7 ± 4.4	0.104
BMI (kg/m <sup>2</sup> )	29.7 ± 2.9	24.4 ± 2.2	0.042
FSH (mIU/mL)	5.9 ± 2.0	6.5 ± 1.6	0.448
LH (mIU/mL)	7.9 ± 2.5	4.2 ± 2.4	0.047
AFC	35.0 ± 16.7	12.2 ± 5.2	0.001
DFC	1.8 ± 1.8	1.7 ± 1.2	0.110
Glucose (mg/dl)	91.1 ± 5.6	87.1 ± 4.3	0.121
Insulin (μU/mL)	11.8 ± 4.8	7.1 ± 3.1	0.032
HOMA index	2.6 ± 0.7	1.6 ± 0.6	0.035
TSH (μU/mL)	1.4 ± 0.7	1.5 ± 0.9	0.600
Hemoglobin (g/dl)	12.9 ± 1.1	13.4 ± 0.9	0.212
Total testosterone (ng/ml)	0.7 ± 0.3	0.5 ± 0.8	0.016
Total cholesterol (mg/dl)	172.1 ± 17.8	151.5 ± 19.6	0.168
LDL (mg/dl)	102.9 ± 23.6	96.2 ± 15.7	0.084
HDL (mg/dl)	45.5 ± 10.9	56.8 ± 8.6	0.066
Triglycerid (mg/dl)	139.3 ± 42.9	102.6 ± 37.7	0.016
Systolic BP (mm/Hg)	120.3 ± 6.9	118.6 ± 8.8	0.267
Diastolic BP (mm/Hg)	76.4 ± 6.7	73.9 ± 6.1	0.398

BMI: Body Mass Index; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone; AFC: Antral Follicle Count; DFC: Dominant Follicle Count; HOMA: Homeostasis Model Assessment of Insulin Resistance; TSH: Thyroid-stimulating Hormone; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein; BP: Blood Pressure. All variables are given mean ± SD. P value obtained by unpaired t test. Two-tailed P value <0.05 was considered statistically significant.

**Table 1:** The demographic, hormonal and biochemical parameters of the PCOS and control groups.

	Patients with PCOS			P	Control			P
	Menstrual day	Ovulatory day	Midluteal day		Menstrual day	Ovulatory day	Midluteal day	
Estradiol (Pg/ml)	43.1 ± 10.9	415.1 ± 475.9 <sup>m</sup>	333.1 ± 265.9 <sup>o,m</sup>	0.001	35 ± 10.5	502.0 ± 515.5 <sup>m</sup>	415.0 ± 375.5 <sup>o,m</sup>	0.001
LF power (ms <sup>2</sup> )	787.8 ± 174.5	753.8 ± 210.0	677.3 ± 206.4	0.159	986.0 ± 251.7	843.0 ± 242.7 <sup>m</sup>	826.0 ± 174.7	0.003
HF power (ms <sup>2</sup> )	281.2 ± 115.4	272.2 ± 73.4	215.2 ± 67.4	0.057	564.2 ± 151.7	419.1 ± 123.7 <sup>m</sup>	480.9 ± 131.7	0.003
LF/HF	2.8 ± 0.9	2.8 ± 1.2	3.3 ± 1.5	0.245	2.0 ± 0.8	2.3 ± 1.0	1.9 ± 0.7	0.738
VLF power (ms <sup>2</sup> )	2182.2 ± 808.8	2126.0 ± 652.8	2071.8 ± 801.8	0.367	2824.2 ± 844.5	2210.2 ± 729.0 <sup>m</sup>	2330.2 ± 625.8 <sup>m</sup>	0.016
Total spectral power (ms <sup>2</sup> )	3199.9 ± 8363.7	3127.2 ± 978.7	2962.2 ± 951.7	0.342	4410.1 ± 834.5	3501.1 ± 979.5 <sup>m</sup>	3705.1 ± 954.5 <sup>m</sup>	0.006
SDNN (ms)	139.4 ± 29.5	126.5 ± 23.5	106.5 ± 25.5	0.022	155.0 ± 21.3	131.0 ± 18.1 <sup>m</sup>	129.2 ± 16.1 <sup>m</sup>	0.006
SDANN (ms)	119.5 ± 19.2	114.5 ± 26.2	104.5 ± 15.6	0.117	137.0 ± 21.6	114.0 ± 17.2 <sup>m</sup>	113.5 ± 17.7 <sup>m</sup>	0.023
SDNN index (ms)	56.1 ± 8.3	55.1 ± 9.2	57.1 ± 12.8	0.784	67.3 ± 7.1	60.3 ± 9.1 <sup>m</sup>	61.6 ± 8.1 <sup>m</sup>	0.008
RMSSD (ms)	31.0 ± 10.2	30.0 ± 7.2	30.0 ± 6.6	0.843	43.3 ± 15.5	37.3 ± 10.5 <sup>m</sup>	40.6 ± 11.5	0.004
pNN50 (ms)	10.9 ± 6.7	9.2 ± 4.7	9.2 ± 3.6	0.487	20.5 ± 11.5	14.5 ± 9.5 <sup>m</sup>	17.3 ± 8.1	0.006
Mean Heart Rate (BPM)	82.1 ± 7.1	83.1 ± 6.2	84.1 ± 2.5	0.095	77.7 ± 5.8	80.2 ± 6.3	78.0 ± 4.9	0.069

LF: Low Frequency; HF: High Frequency; VLF: Very Low Frequency; SDNN: the Standard Deviation (SD) of all R-R intervals; SDANN: the SD of the Averages of R-R intervals during all 5-min periods that constitute the 24-h day; RMSSD: the Root Mean Square of Successive Differences; pNN50: percent of R-R intervals differing more than 50 ms from each other; SDNN index: mean of the Standard Deviations of all R-R intervals for all 5-min segments of the 24-h recording; BPM, Beat Per Minute; All variables are given mean ± SD. Two-way Analysis of Variance was used for the analysis of the data between the three phases of the cycle, and Wilcoxon Signed Ranks Test was used for the comparison of the means of the two groups which are dependent. Two-tailed P value <.05 was considered statistically significant. <sup>m</sup> vs menstrual day P <0.05, <sup>o</sup> vs Ovulatory day P <0.05.

**Table 2:** The Heart rate variability indices in the PCOS and control groups according to the phases of the ovulation induction cycle.

	Menstrual day		P	Midluteal day		P
	Patients with PCOS	Control		Patients with PCOS	Control	
Estradiol (Pg/ml)	43.1 ± 10.9	35 ± 10.5	0.623	333.1 ± 265.9	415.0 ± 375.5	0.148
Progesterone (ng/ml)	0.2 ± 0.2	0.1 ± 0.3	0.536	25.2 ± 26.5	31.5 ± 39.9	0.516
LF power (ms <sup>2</sup> )	787.8 ± 174.5	986.0 ± 251.7	0.030	677.3 ± 206.4	826.1 ± 174.7	0.009
HF power (ms <sup>2</sup> )	281.2 ± 115.4	564.5 ± 151.7	0.004	215.2 ± 67.4	480.9 ± 131.7	0.001
LF/HF	2.8 ± 0.9	2.0 ± 0.8	0.018	3.3 ± 1.5	1.9 ± 0.7	0.004
VLF power (ms <sup>2</sup> )	2182.2 ± 808.8	2824.2 ± 844.5	0.075	2071.8 ± 801.8	2330.2 ± 625.8	0.400
Total spectral power (ms <sup>2</sup> )	3199.9 ± 8363.7	4410.1 ± 834.5	0.056	2962.2 ± 951.7	3705.1 ± 954.5	0.044
SDNN (ms)	139.4 ± 29.5	155.0 ± 21.3	0.071	106.5 ± 25.5	129.2 ± 16.1	0.034
SDANN (ms)	119.5 ± 19.2	137.0 ± 21.6	0.107	104.5 ± 15.6	113.5 ± 17.7	0.289
SDNN index (ms)	56.1 ± 8.3	67.3 ± 7.1	0.035	57.1 ± 12.8	61.1 ± 8.1	0.201
RMSSD (ms)	31.8 ± 10.2	43.3 ± 15.5	0.012	30.0 ± 6.6	40.6 ± 11.5	0.049
pNN50 (ms)	10.9 ± 6.7	20.5 ± 11.5	0.017	9.2 ± 3.6	17.3 ± 8.1	0.043
Mean Heart Rate (BPM)	82.1 ± 7.1	77.7 ± 5.8	0.094	84.1 ± 2.5	78.0 ± 4.9	0.004

All variables are given mean ± SD. The variables were analyzed by Mann-Whitney U test. Two-tailed P value <0.05 was considered statistically significant

**Table 3:** The comparison of HRV indices in the menstrual day and midluteal day of ovulation induction cycle between PCOS and control group.

a problem linked with long-term health risks such as metabolic syndrome and cardiovascular disorders [16]. Despite a wide range of studies on the pathogenesis of PCOS, there is still an ongoing debate about the mechanism underlying it. The anomalies detected in PCOS can be traced down to a number of causes: the primary ones are hyperinsulinemia, insulin secretion leading to insulin resistance, and the defect under the effect of insulin. Also playing a role in the pathophysiology are the neuroendocrine defect, which causes an exaggerated LH pulse frequency and amplitude; the defect in androgen synthesis, which increases the production of ovarian androgen; and finally the change in the cortisol metabolism, which leads to increased production of adrenal androgen [17]. It is argued that sympathetic activity and stress play a large role in ovarian pathologies that affect the reproductive functions [2]. The sympathetic nerve is necessary for the normal function of the ovary. Stresses, and the sympathetic over activation caused by stress, are accepted as an etiological factor in cystic ovarian pathologies of anovulatory nature [2].

Other studies shown that women with PCOS have higher levels of stress and sympathetic nerve activity [2,18]. Hypothalamic and intraovarian mechanisms play a role in sympathetic overactivity. Stress assumes a role in the pathogenesis of PCOS by causing sympathetic discharge from the paraventricular nucleus [19]. There is a link between stress and increased steroid secretion. Anxiety is a component of the response to stress and cortisol regulates this response. An increase has been detected in all the anxiety symptoms observed in women with PCOS [20]. These mechanisms contributing to the genesis and continuance of PCOS might be related with the ovulation induction problems that arise in the syndrome.

Higher levels of cortisol in the follicle cyst have been found to be linked with the increased response to HCG. It is considered that the high level of sympathetic activity renders the follicle cyst hypersensitive to HCG [21]. The ovaries of patients with PCOS contain more catecholaminergic nerve fibers than normal ovaries [22]. The scission of the catecholaminergic nerve fibers of the ovarian cells decreases the exaggerated ovarian response resulting from gonadotropins and beta-adrenergic stimulation [23]. Women with PCOS have a higher level of sympathetic nerve activity (as detected through lower HRV measurements) than the women in the control group. In our study, we observed that the sympathetic predominance that developed in the luteal phase of the induced cycles of normal women did not occur in the induced cycles of women with PCOS. The function of the reproductive system is effected by the ANS. There might be a link between the ovulatory dysfunction in PCOS and autonomic dysregulation. However, further studies are needed to determine whether this is a cause or a result.

According to the findings of the study, ovulation induction treatments appear to be linked with a reduction in the parasympathetic tonus and a relative increase in the sympathetic tonus. This means that ovulation induction treatments for the infertile population have the risk of leading to undesirable cardiovascular consequences. These patients should be monitored closely during their treatment, and the risk in question must always be kept in mind. Again, further studies are needed to clarify this issue.

A link has been found in obese patients between insulin resistance and increased sympathetic nerve activity [24]. The negative link of the cardiac vagal tonus with insulin resistance has also been demonstrated [25,26]. Between 50-70% of the patients with PCOS have varying degrees of insulin resistance [27]. Accordingly, insulin resistance may also be contributing to the increased sympathetic activity in PCOS.

The women with PCOS who took part in this study turned out to have higher HOMA index values, which indicate insulin resistance. However, no correlation was detected between the HOMA index and the HRV measurements.

HRV, which has been widely used for the last two decades, is accepted as a sound and noninvasive indicator of the autonomic balance [28]. Defined as the cyclical changes in the sinus rate through time, HRV provides information about the sympathetic-parasympathetic balance. HRV is generally linked with vagal mechanisms [28]. The low level of the time domain measurements is related with reduced parasympathetic activity. Among the frequency domain measurements, LF shows the sympathetic and HF the parasympathetic activity [29]. The time and frequency-domain parameters of HRV mirror to vagal activity, but not detect sympathetic activity according to other researcher [30].

The luteal phase of the menstrual cycle is linked with an increase in the LF component and a decrease in the HF component [8,12]. This situation shows that the sympathetic nerve activity is predominant in the luteal phase. It was observed in the study that the HRV measurements dropped on the midluteal period and the sympathovagal balance shifted towards the sympathetic side in the control group. These observations agree with those made in studies on the natural menstrual cycle. Very few studies in the literature show the effects of ovulation induction on the HRV indices. It has been argued that the sudden and excessive rise in the estrogen levels of women treated with ovulation induction for the purpose of in vitro fertilization leads to vagal activation, and is linked with variable sympathovagal balance [31]. The patients in our study were treated with a mild ovulation induction and there was a correspondingly mild increase in their estrogen levels.

### The limitations of the study

The patients we selected for inclusion in this study were oligo/amenorrheic infertile women with PCOS. According to the Rotterdam definition, some women with PCOS do not have any problems related with ovulation or infertility. Therefore, these results cannot be generalized for all patients with PCOS. Nor does this study provide information about the intra-cycle ANS variations of those PCOS patients with spontaneous ovulation. Moreover, all infertile women have high levels of anxiety, the exact amount varying with respect to the duration of infertility and whether they have suffered miscarriage [32]. But women with PCOS have already high anxiety levels [33]. Therefore, having both infertility and PCOS, as was the case with the patients in our study, may have led to excessive levels of anxiety and consequently given rise to exaggerated results. The changes in HRV might have been evaluated using the anxiety scale, but we did not undertake such an evaluation in the present study.

In conclusion, this study shows that anovulatory women with PCOS have reduced cardiac autonomic modulation throughout ovulation induction cycles. There might be a link between the disturbed ovulation processes in PCOS and the reduced intra-cycle HRV fluctuation.

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