Correlation Between Hormonal Parameters and Ultrasonographic Appearance in Polycystic Ovary Syndrome

POÜKİSTİK OVER SENDROMUNDAKİ HORMONAL PARAMETRELER İLE ULTRASONOGRAFİK GÖRÜNÜM ARASINDAKİ KORELASYON

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Summary

Objective: *The aim of this study was to investigate the relationship between the ultrasonic findings of polycystic ovary (PCO) and endocrine milieu in anovulatory women with polycystic ovary syndrome (PCOS) and in ovulatory controls.*

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Materials and Metods: *Endocrine parameters and sonographic ovarian morphologic features were evaluated in 57 patients with PCOS and in 20 normal ovulatory women.*

Results: *The most appropriate linear predictions for serum total and free testosterone, androstenodionc and LH levels were achieved by using the number of microcvst, microcyst's volume and stromal and ovarian volume, as follows: (I) total testosterone--J(0.064 x stromal volume) + (0.012 x ovarian votume) + 0.59J, (R2=0.72), (2) free testosterone-[(0.79 x stromal volume) +16.85], (R2—Ù.60), (3) androstenodione~—[(2.76 x microcyst volume)+(0.17 x stromal volume)-(0.04 x ovarian volume)+2.44], (R2=0.61), (4) LH=(2.88 x stromal volume)+20.56. (R2=0.60), p < 0.001.*

Conclusion: *The results suggest that the evaluation of ovarian morphologic features provides valuable information about the endocrine parameters of polycystic ovary syndrome and the diagnosis and the treatment modalities of polycystic ovaiy syndrome and may also be used during basic infertility in vestigations.*

Key Words: Polycystic ovary, Polycystic ovary syndrome, Ovarian ultrasonography

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Ozet

Amaç: *Bu çalışmanın amacı polikistik över sendronu olan kadınlar ile ovu/atuvar kontrol grubunda polikistik överin ultrasonografik bulguları ile endokrin parametrelerin ilişkisini incelemektir*

Çalışmanın yapıldığı yer: *Cumhuriyet Üniversitesi Tıp Fakültesi Kadın Hastalıkları ve Doğum Anabiliri! Dalı, Sivas.*

Materyal ve Metod: *Endokrin parametreler ve sonogrofik ovarian morfolojik bulgular 57polikistik över sendromlu ve 20 ovulaluvar kadında değerlendirildi.*

Bulgular: *Serum total ve serbest testosteronu, androstenodion ve LH düzeylerini en iyi öngören doğrusal ilişki mikrokist sayısı, mikrokistlerin volümii ve stromal ve ovarian volüm kullanılarak aşağıdaki şekilde elde edildi: (1) total testosteron=[(0.064 x stromal voliin)+(0.012 x ovarian volüm)+().59J, (R2--=0.72), (2) serbest testosterondu 0.79 x stromal volüm) +16.85], (R2=0.60), (3) androstenodion--=[(2.76 x mikrokist volümii)+(0.1'7 x stromal voliin)-(0.04 x ovarian volüm)+2.44], (R2=0.61). (4) LH=(2,88 x stromal volüm)+20.56, (R2==0.60), p < 0.001.*

Sonuç: *Bulgularımız, överin morfolojik özelliklerinin değerlendirilmesinin polikistik över sendromunun endokrin bulguları ve polikistik över sendromunun tanı ve tedavi yöntemleri açısından önemli bilgiler sağlayabileceğini ve temel infertilité araştırmaları sırasında kullanılabileceğini öngörmektedir.*

Anahtar Kelimeler: Polikistik över, Polikistik över sendronu, Overian ultrasonografi

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Polycystic ovary syndrome (PCOS) is an association of oligomenorrhoea, anovulation, hyperandrogcnısm, hypcrmsulınemıa, gonadotropin sccrction abnormalities, truncal obesity, hirsutism and enlarged polycystic ovaries. This syndrome is clin-

ically and biochemically heterogenous but excessive androgen production is characteristic.

Ultrasound characterization of ovarian morphology has become an important aspect of gynecological endocrinology (1). Using laparoscopic inspection as a reference test, the diagnostic sensitivity and specificity of ultrasound in PCOS have been 91% and 100% respectively (2). Poison et al have found that over %90 of women with PCO (polycystic ovary) had a clinical or biochemical feature consistent with the ultrasound diagnosis (3). However, ultrasonograpy alone cannot be used for the diagnosis of PCOS because of the heterogenecity of the syndrome. The descriptions have been made as follows :

PCO should be diagnosed when more than eight discrete follicles of smaller than 10 mm diameter are seen in the ovary, usually peripherally arrayed around an enlarged, hyperechogenic, central stroma (4). PCOS is the typical ultrasound features of the polycystic ovary associated with oligo/amenorrhoea and/or clinical symptoms of hyperandrogenism, such as hirsutism or acne (5).

We investigated the relationship between the ultrasonographic appearance of the ovaries and the hormonal parameters in anovulatory women with PCOS, and compared these findings with the ovulatory normal women.

Materials and methods

In order to investigate the pathological characteristics of polycystic ovaries (8 cysts<) diagnosed by transabdominal or transvaginal ultrasound (TVS) in patients with PCOS (n=57), the relationship between morphological and endocrine changes were studied and ovulatory women served as controls (n=20). Sonographic parameters of each ovary were assessed and the mean values of them were taken into account. The polycystic ovary must be distinguished from normal ovaries and, more importantly, multifollicular ovaries. The latter, which are also enlarged and multicystic, but characteristically have minimal stromal tissue (4). Owing to inability in assessing the stromal component of multifollicular ovary (MFO), these cases were exclude from the study. The relationship between the number of small cyst, microcyst diameter and volume, stromal cchogcnecity length and volume, ovarian length and volume and serum total testosterone, free testosterone, androstenedione (ASD), DHEA-S, 17-a OHP (17a OH progesterone), LH (luteimsing hormone), FSH (follicle-stimulating hormone), SHBG (sex hormone binding globulin), GH (growth hormone), insulin, prolactin, estrone, estradiol and progesterone levels were examined and their association with Ferriman-Gallwey score (6) and BMI (body mass index) was also explored. Ovulation was assessed by midluteal (cycle day 21) serum progesterone level (cut-off 2.5 ng/ml), BBT (basal body temparature) chart and follicular measurements on ultrasonography.

Patient selection

Based on the criteria of Adams et al (4) , PCO should be diagnosed when more than eight discrete follicles of <10 mm diameter are seen in the ovary, usually peripherally arrayed around an enlarged, hyperechogenic, central stroma. Each patient had a history of menstrual disturbance, participated in cither an infertility or hirsutism treatment program, and more than 8 small cysts in each ovary detected by sonography. Ovarian ultrasound appearance was classified as a peripheral cystic pattern or general cystic pattern according to Ardaens et al (7).The two ethiologic entities polycystic ovaries (PCO) and multifollicular ovaries (MCO) are defined as the follicles are peripherally arrayed in both ovaries in the first, and dispersed in whole ovarian tissue in the second. Due to inability in assessing the size of the stroma, patients who had general cystic pattern on ultrasound were excluded from the study. The ovarian volume was calculated as 1/2 x dlxd2xd3 (dl=maximal transverse diameter; d2=maximal longitudinal diameter; d3=maximal anteroposterior diameter) (8). An ovarian volume of 8.44 cm^3 or 6.2 ml according to Puzigaca et al (9) or Takahashi et al (10) were both used as a cut-off value for normal ovarian size, respectively.

Hormonal assay

Hormonal evaluations included measurement of estradiol, estrone, progesterone, LH, FSH, total serum testosterone, free testosterone, ASD, DHEA-S, Prolactin, SHBG, GH, insulin. Blood samples were taken in the early follicular phase (between days 2 and 5) of the cycle. Hormonal measurements were carried out by EIA (enzymimmuno assay) and

RIA (assay) using commercially available kits. EIA from DPC (Diagnostic Product Cooporation) was used for estradiol, progesterone, FSH, LH, prolactin, GH. Kodak was used for estrone, DPC was used for insulin and free testosterone, Spectra was used for SHBG and total testosterone, and DSL (Diagnostic System Laboratories) was used for ASD by RIA,

A Combison 410 Kretz ultrasound machine (Tiefcnbach, Austria) with a 7.5 MHz transvaginal and 3.5 MHz transabdominal probes were used for the scans.

Hirsutism was defined according to the criteria of Ferriman and Gallwey who graded nine hormone-sensitive body areas from 0 and 4. Scores of less than 8 were considered nonhirsute.

Adiposity was assessed by the body mass index (BMI: kg/in?).

Statistics

The data were described by the mean and the range. A Mann-Whitney test for unpaired data was applied for comparison of two groups. The tests were all performed two-sided at the 5 % level of significance with Bonferroni's correction. The relation between continuous variables was evaluated by means of the Pearson's correlation coefficient and by means of linear regression. Multiple regression analysis was performed in order to evaluate the impact of the combination of ovarian morphologic features on the level of endocrine parameters of PCOS. Statistical analysis were carried out with the SPSS for Windows program.

Results

The differences between the patients and the controls in regard to mean age, BMI, FG score, estradiol, estrone, LH, FSH, LH/FSH, progesterone, f.testosterone, t.testosterone, DHEA-S, 17-a OHP, androstenedione, SHBG, GH, insulin, prolactin, microcyst number and volume, stromal volume and ovarian volume were shown in Table 1.

Correlations between endocrine parameters and ovarian morphology

As reported in Table 2, ovarian volume had the best correlation with total testosterone in patients (r=0.75, pO.001). High correlations were found

Table 1. Comparison of the mean with SD and ran<*e of the patient and the control groups

	Patient (57)			Control (20)			
Parameter	mean	SD	range	mean	SD	range	P
Age	23.99	4.48	17-34	22.3	3.82	17-29	0.160
BMI	26.50	2.99	20.5-34.4	25.55	3.02	21.3-30.2	0.225
FG Score	16.72	3.09	10-27	-	-	-	-
Estradiol (pg/ml)	58.85	28.14	19.1-127.3	50.21	16.82	24.9-84.6	0.201
Estrone (pg/ml)	56.77	25.28	20.2-115.4	34.77	8.33	23.3-50.4	<0.001
LH (mIU/ml)	26.47	6.32	17.2-45.7	3.97	0.95	2.3-5.9	<0.()01
FSH (IU/L)	8.70	1.68	6.2-12.6	4.31	1.38	2.3-7.9	<0.0()l
LH/FSH	3.01	0.92	2.02-5.52	0.94	0.24	0.5-1.76	<0.001
Progesterone (ng/ml)	0.39	0.14	0.27-0.85	0.72	0.35	0.2-1.5	<0.001
F.Testosterone (pg/ml)	18.47	1.72	15.5-22.1	4.23	1.04	2.4-6.2	<0.001
T.Testosterone (ng/ml)	0.88	0.17	0.64-1.22	0.42	0.13	0.21-0.7	<0.001
DHEA-S (pg/ml)	3.70	0.89	2.6-5.7	3.49	0.69	2.10-5.20	0.341
17 a OH Progesterone (ng/ml)	1.47	1.08	0.48-5.23	0.47	0.16	0.24-0.75	<0.001
Androstenedione (ng/ml)	2.97	0.56	2.1-4.3	1.56	0.49	0.8-2.4	O.001
SHBG (nmol/L)	24.07	6.50	17.1-42.4	53.36	26.02	21.8-105.3	<0.001
GH (ng/ml)	4.02	2.35	0.27-9.2	3.76	2.42	0.80-8.20	0.675
Insulin (pIU/ml)	11.01	2.19	7.1-14.7	9.91	2.45	6.3-14	0.064
Prolactin (ng/ml)	13.7	4.04	5.5-19.8	12.10	3.82	6.6-19	0.136
Microcyst Number	14.28	2.86	8-21	4.45	1.46	2.5-7	<0.001
Microcsyt Volume (cm^3)	0.25	0.10	0.13-0.52	0.77	0.49	0.12-1.84	<0.001
Stromal Volume (cm^3)	2.05	1.70	0.3-6.52	-	-	-	-
Ovarian Volume (cm^3)	12.06	3.9	5.9-21.98	4.44	1.04	2.75-6.24	<0.001

FG score: Ferriman-Gallwey score; Mann-Whitney U nonparametric test; statistically significant difference between means: Bonferroni's test P< 0.002 was considered significant.

Table 2. Correlations between endocrine parameters, **BMI,** FG score and ovarian morphology

	Microcyst number	Microcyst volume (enr')	Stromal Volume (cm'')	Ovarian Volume (cm)
F.Testosterone (pg/ml)	0.34[c]	0.58[d]	0.78[d]	0.63[d]
T.Testoslerone (ng/ml)	0.33"	0.58[d]	0.83[d]	[illegible] d
Androstenedione (ng/ml)	0.19"	0.73[d]	0.65[d]	0.35[c]
DHEA-S (pg/ml)	0.002"	0.23"	0.19"	0.20"
17 ocOHP (ng/ml)	0.43[c]	0.70[d]	0.86[d]	0.60[d]
FSH (IU/L)	0.15"	0.09"	0.04"	0.16"
LH (mlU/ml)	0.32[b]	0.52[d]	0.77[d]	0.49[d]
LH/FSH	0.12"	0.15"	0.37[c]	0.22"
Estradiol (pg/ml)	0.22"	0.10"	0.34[b]	0.33[b]
Estrone (pg/ml)	0.33[b]	0.28[b]	0.54[d]	0.42[c]
Progesterone (ng/ml)	-0.26"	0.13"	-0.02"	-0.19"
SHBG (nmol/L)	-0.3 I[h]	-0.46[d]	-0.57[d]	-0.55[d]
Insulin (plU/ml)	0.009[11]	-0.10"	-0.04"	-0.02"
GH (ng/ml)	0.06"	0.14"	-0.08"	-0.15"
Prolactin (ng/ml)	-0.12"	0.19"	0.10"	-0.01"
Progesterone (ng/ml)	-0.26"	0.13"	-0.02"	-0.19"
BMI	0.12"	0.05"	0.20"	*0.33*[b]
FG Score	0.34[c]	0.37[c]	0.45[d]	0.45[d]

Values with the same superscript were significantly different: "p> 0.05; [b]p<0.05; [c]p<0.01; [d]p0.001.

for ASD and microcyst and stromal volume (r=0.73, r=0.65, p<0.001, respectively). 17-a OHP had the best correlation with stromal volume (r=0.86, p<0.001). Serum estrone levels had stronger correlations than serum estradiol levels with ovarian morphologic features.

Correlations between Ferriman-Gallwcy score, BMI and ovarian morphology

The amount of stroma had the highest negative correlation with serum SHBG levels (r=-0.57, p<0.001). Strong correlations were noted both between hirsutism scores and stromal and ovarian sizes (r=0.45, p<0.001). BMI was positively correlated with ovarian volume (r=0.33; p<0.05). A11 were listed in Table 2.

In the analysition of the control group, no significant correlation was computed among those mentioned above.

Assessing the strength of the linear relationship between two variables, the strong statistical significance (p< 0.001) and the great magnitude of the correlation coefficient (r>0.75) was identified between: (i) total testosterone and stromal and ovarian volume (r=0.83, r=0.75, respectively), (Figure 1), (ii) androstenedione and microcyst volume (r=0.73), (Figure 2), (iii) LH and stromal volume (r=0.77), (Figure 3), pO.001.

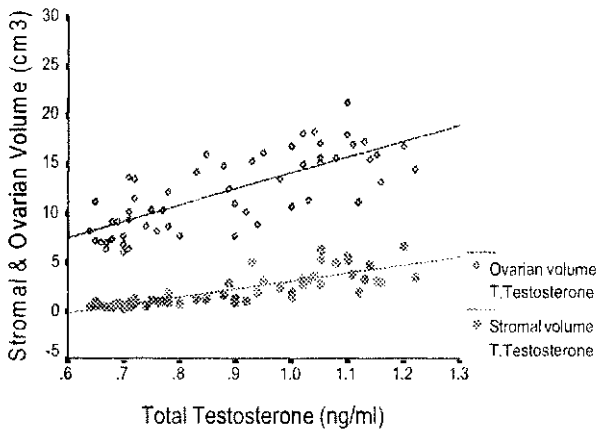

Figure **1.** Correlations with serum total testosterone levels and stromal echogenecity and ovarian volume in women with PCOS.

The most appropriate linear predictions for the endocrine parameters of PCOS were made as follows: (1) total testosterone=[(0.064 x stromal volume)+(0.012 x ovarian volume)+0.59], (RM).72), (2) free testosterone=[(0.79xstromal volume)+ 16.85], (R2=0.60), (3) androstenodionc=[(2.76 x microcyst volume)+(0.17 x stromal volume)-(0.()4 x ovarian volume)+2.44], (RMJ.61), (4) LH=(2.88

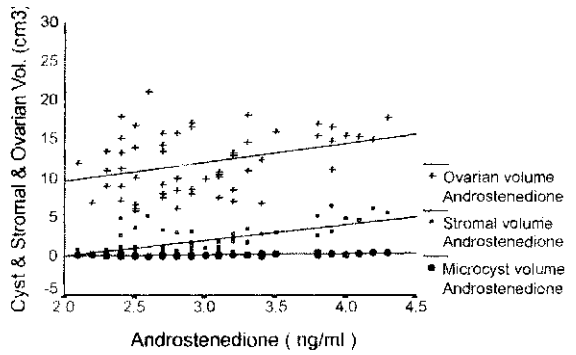

Figure 2. Correlations with serum androstenedione levels and microcyst, stromal and ovarian volume in women with PCOS.

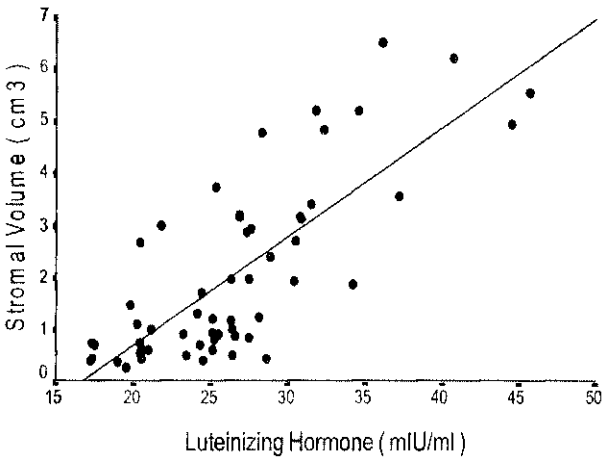

Figure 3. Correlation with LH levels and stromal echogenicity volume in women with PCOS.

x stromal volume)+20.56, (R^2=0.60), (by the stepwise method of multiple regression analysis).

Discussion

Evaluation of morphology in polycystic ovaries may reveal endocrine abnormalities and facilitate selection of proper treatment (11). In our study, in patients with PCOS, a significant positive correlation was noted between serum total testosterone and the number of small cysts and microcyst, stromal and ovarian volume, in which stromal echogenecity volume had the greatest positive correlation with total testosterone. The most suitable estimation for serum total testosterone was obtained by using stromal and ovarian volume. LH levels were higher in the anovulatory PCOS than ovulatory PCOS (12). Ehrmann et al ascribed the hyperactivity of 17 a-OHP to desensitization of the LH receptor by the sustained high LH levels observed in this syndrome (13). Takahashi et al have shown no significant correlations between the increases in the number of ovarian microcysts and gonadotropin levels in ovulatory patients with MFO, however, estrone and androgen levels tended to increase as the number of cysts increased (14). It is suggested that an increase in intra-ovarian small cysts leads to increased production of ovarian androgen, in turn influencing the secretion of gonadotropin (15). Pache et al have found significant correlation between increased LH and testosterone levels, and increased number of small sized follicules (16). In the current study, both serum LH and estrone levels were significantly related with the number of microcyst. A linear prediction can be made for serum LH level by using stromal echogenecity volume. Some studies have shown that the mean microcyst diameter is significantly higher in the PCOS patients than the controls (17). In our study, serum 17a-OHP and ASD had the best correlation with microcyst volume. It has been demonstrated that serum 17 a-OHP levels are elevated in patients with PCOS compared with normal women (18) and anovulatory women with PCOS has an abnormal regulation of 17-hydroxylase and 17,20-lyase activity in the ovary and 17 a-OHP is formed in excess (12, 19). ASD source is predominantly ovarian .

Ovarian stromal hypertrophy is a frequent and specific feature of hyperandrogenism. It correlates with the ovarian androgenic dysfunction (20). Thecal and stromal cells are responsible for luteinizing hormone-dependent androgen production and may be hyperplastic as a result of increased LH release characteristic of hyperandrogenic ovulation (21). Pache et al have found significant correlation between increased LH and testosterone levels and increased amount of ovarian stroma (16). Dewailly-P et al have suggested that elevated serum immunoreactive LH levels was commonly but not always linked with the ovarian stromal increment. In our study, stromal echogenecity volume were positively correlated with serum total testosterone, free testosterone, ASD, 17 a-OHP, LH, estrone, estradiol levels and LH/FSH ratios, with the greatest correlation was detected in serum total testosterone and 17 a-OHP levels. Dewailly et

al have described an objective quantitative method of ovarian stromal assessment by using a computerized ultrasound technique to measure stromal and microcyst areas (20). They found that the stromal area in hyperandrogenemic women was significantly larger than in those with nonnal androgen levels. In hyperandrogenemic women, they have also found that the stromal area correlated with serum ASD and 17 a-OHP concentrations but not with basal serum testosterone, LH or insulin concentrations. Although by using computerized ultrasound technique, a much more objective, standardized diagnosis of PCO can be obtained, but this may not be cost-effective.

Women with PCO had a greater mean ovarian volume, regardless of hormonal contraception use (22). The mean ovarian volume has been significantly higher than the normal ovulatory women (17). In the current study, forty-four (78%) women with PCOS had an enlarged ovarian volume greater than 8.44 cm^3, on the other hand, all of the ovaries of the 57 (100%) PCOS patients were larger than 6.2 ml. Thus it seems logical to accept the ovarian volume of 6.2 ml as a cut-off value for the ovarian size of ovulatory normal women. However to state clearly, polycystic ovaries are usually but not invariably enlarged and typical features of normal size ovaries do not preclude diagnosis. In our study, ovarian volume had a significant positive correlation with serum total and free testosterone, ASD, 17 a-OHP, LH and estrone levels with the greatest correlation was noted between total testosterone and ovarian volume. Edun et al have found an association between increased ovarian volume and serum testosterone and LH levels, LH/FSH ratio, and free androgen index (22, 23). Pache et al have found a significant correlation between augmented ovarian volume and increased LH, and testosterone (12). Results show that linear predictions for serum total and free testosterone, ASD and LH levels can be assessed by using microcyst, stromal and ovarian volume.

Estrogen secretion in PCOS has been characterized by circulating estrone levels (25). In our study, serum estrone levels had closer correlations than serum estradiol levels with ovarian morphologic features. The reversal of the normal circulating estrone- estradiol ratio reflects the impact of extraglandular aromatization of ASD and testosterone. Since ASD is the predominant ovarian androgen, conversion to estrogen is more pronounced than that of testosterone to estradiol.

Insulin and insulin resistance provides additional predictive value for ovarian volume and stromal echogenecity (16) . Stromal hyperthecosis is present in PCOS women with moderate hyperinsulinemia (26). A direct correlation has been identified between circulating insulin levels and the presence and extent of stromal hyperthecosis (27). We observed no correlation between the stromal amount and serum insulin levels. Most evidence suggests that insulin amplifies androgen production and may influence gonadotropin secretion (28). In vitro studies have shown that insulin or insulin-like growth factor I can enhance the ovarian androgen response to gonadotropin stimulation (29,30). Polycystic ovarian changes and stromal hyperthecosis may be appear to be a part of the same morphologic process, and hyperinsulinemia may have an additional role in predicting testosterone value for ovarian volume and stromal echogenecity but the amount of stromal hypertrophy seems to be not have a direct relationship with the severity of hyperinsulinemia.

The PCOS is commonly associated with obesity and that subset of women shows a much higher incidence of hirsutism (31). In the presenting study, forty-three (75.43%) women with PCOS were in the obese range (BMI>or=25 kg/m2). There was a strong correlation between Ferriman-Gallwey scores and BMI in our PCOS patients. Falsetti et al have demonstrated high hirsutismus scores with enlarged ovaries in PCOS patients (17). In our study, hyperandrogenemia was excellently correlated with hirsutism scores, in this regard Ferriman-Gallwey scores had significantly positive correlations with all of ovarian morphologic features and the best coefficients were obtained in the stromal and ovarian size measurements.

In summary, microcyst, stromal and ovarian volume are the most powerful sonograpyhic ovarian morphologic features in predicting the serum assays of total and free testosterone, ASD and LH. Assessment of the ovarian morphologic features with sonography can be a valuable additional tool for the diagnosis and the selection of proper treatment of PCOS, and may be helpful in determining severity of this syndrome. Evaluation of these im-

ages may also be used during basic infertility investigations. Their more detailed specification is **a** task for the future.

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