



Evaluation of metabolic and hormonal parameters in women with PCOS living in Black Sea Region

Nur DOKUZEYLÜL GÜNGÖR^{1,*}, Samettin ÇELİK², Mehmet GÜÇLÜ³, Sebahattin ÇELİK⁴, Ferhat CENGİZ⁵

¹Department of Reproductive Endocrinology and IVF, Medical Park Göztepe Hospital, Bahçeşehir University, Istanbul, Turkey

²Department of Obstetrics and Gynecology, Samsun Education and Research Hospital, Samsun, Turkey

³Department of Obstetrics and Gynecology, Pendik Education and Research Hospital, Marmara University, Istanbul, Turkey

⁴Department of Obstetrics and Gynecology, Balıkesir State Hospital, Balıkesir, Turkey

⁵Department of Histology and Embryology, Medical Park Göztepe Hospital, Bahçeşehir University, Istanbul, Turkey

Received: 07.09.2020

Accepted/Published Online: 07.02.2021

Final Version: 23.04.2021

Abstract

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy which is frequently associated with metabolic syndrome. The aim of this study is to compare the clinical, biochemical and hormonal characteristics of healthy women and women with PCOS in Black Sea Region. 91 healthy women (Group 1) and 109 PCOS (Group 2) patients were included in this retrospective study. This study was conducted in Samsun Women and Children's Health Research and Training Hospital between October 2019-May 2020. The anthropometric, clinical and laboratory characteristics of the women were recorded. The Institutional Review Board of the hospital approved the study. IBM SPSS Statistics 22 program was used for statistical analysis. Variables were expressed as mean \pm standard deviation or as a number (percentage), and statistical significance was defined as a p value of less than 0.05. In this study, no statistically significant difference was found between patients with PCOS and control subjects in age, height, BMI, waist circumference and waist-to-hip ratio. Weight, BP systolic, BP diastolic and FG scores, were significantly higher in patients with PCOS compared to control subjects. In this study, no statistically significant difference was found between patients with PCOS and control subjects in FSH, estradiol prolactin, TSH, ft3, ft4 and DHEASO4 levels. But, LH, cortisol, total and free testosterone and 17-OH progesteron levels were significantly higher in patients with PCOS compared to control subjects. Also in this study, vitamin B12 and the 25-OH-D levels were significantly lower in patients with PCOS compared to control subjects. HbA1C, fasting blood glucose levels, fasting insulin levels and HOMA-IR were significantly higher in patients with PCOS compared to control subjects. Total-C, LDL-C and TG levels were significantly higher in women with PCOS compared to control subjects. Also, HDL-C levels were significantly lower in women with PCOS compared to control subjects. As a result, the risk of metabolic syndrome is increased in PCOS patients because of high androgen levels, obesity and insulin resistance.

Keywords: hormone profile, lipid profile, metabolic syndrome, polycystic ovarian syndrome

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of reproductive-aged women. Although Stein and Leventhal first described it in 1935, its etiology is still unclear (Stein and Leventhal, 1931). Menstrual irregularities, hyperandrogenism, hirsutism, and fertility problems are the main clinical features of PCOS. The prevalence of PCOS is 5-10%, and it depends on environmental and genetic factors and ethnicity (Abbot et al., 2002). This syndrome's diagnosis requires the exclusion of manifestations with similar clinical presentation, such as congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumors, and other causes of androgen excess. Three available criteria used to diagnose PCOS: (i) the 1990 National Institutes of Health (NIH) and National Institute

of Child Health and Human Development (NICHD) criteria; (ii) the 2003 Rotterdam criteria; and (iii) the 2006 Androgen Excess Society criteria. The most widely used one is Rotterdam criteria, and according to the 2003 Rotterdam criteria, it is usually defined by the presence of at least two out of the following three features: i) oligoovulation or anovulation, ii) clinical and biochemical signs of hyperandrogenism (presence of at least two signs) or iii) polycystic ovaries (ovaries with many small cysts no larger than 8-10 mm) (Rotterdam ESHRE/ASRM, 2004). The increasing levels of luteinizing hormone (LH) during the menstrual cycle is responsible for several changes, including the synthesis of androgens by the theca cells, resulting in hirsutism, acne, and androgenic alopecia. Most PCOS patients also present with peripheral

insulin resistance (IR) and hyperinsulinemia. Insulin plays a critical role in the clinical manifestations of PCOS by increasing the synthesis of androgens by the theca cells and inhibiting the hepatic sex hormone-binding globulin (SHBG). In this way, it increases the fraction of free testosterone to bind with its receptor. Obesity is also very common in PCOS patients, including a higher waist-hip ratio (visceral obesity). This contributes to the exacerbation of menstrual irregularities and many other metabolic alterations (Poretsky and Piper, 1984). A metabolic syndrome is a group of metabolic disorders including insulin resistance, impaired glucose metabolism, abdominal obesity, hypertension, and dyslipidemia. It increases the risk of Type 2 diabetes mellitus (DMT2), coronary heart disease (CHD) and endometrial cancer. Patients with PCOS are at high risk for cardiovascular diseases because of hyperandrogenism, insulin resistance, type II diabetes, and obesity which are commonly seen in PCOS (Dunaif et al., 1989). Adipose tissue dysfunction contributes to insulin resistance in women with PCOS. However, a substantial number of lean women affected by PCOS have insulin resistance as well, independent of obesity (Ketel et al., 2011). Vitamin B12 is important in the remethylation of homocysteine to methionine, and hyperhomocysteinemia is a characteristic of vitamin B12 deficiency (McCarty, 2000). Insulin resistance in women with PCOS is associated with high plasma homocysteine levels (Schacter et al., 2003). Hyperhomocysteinemia is associated with increased cardiovascular disease risk in type 2 diabetic patients. Vitamin D also plays an essential role in metabolic pathways affected by PCOS, and vitamin D receptor (VDR) polymorphisms are associated with some of the patterns presented by PCOS (Manousopoulou et al., 2015). This study compares the clinical, biochemical, and hormonal characteristics of healthy women and women with PCOS living in the Black Sea Region.

2. Materials and methods

This retrospective, cross-sectional study was conducted with 200 women aged 15-44 years who underwent gynecological examinations in the outpatient clinic of a state hospital between June 2019 and May 2020. This study followed the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Health Sciences University Samsun Research and Education Hospital 2020/15/8. The study was carried out with the permission of the Local Institutional Review Board. Among them, 91 women were healthy volunteers with normal menstrual cycles who had no features of hyperandrogenism and 109 women with the diagnosis of PCOS according to revised Rotterdam Criteria ESHRE/ASRM criteria (2004). All data were abstracted from the electronic medical records, histories, and physicals. A detailed medical, reproductive, and family history had been taken. Also, anthropometric measurements were performed, including height, weight, body mass index (BMI), waist circumference, hip circumference, waist-hip ratio (WHR), systolic and diastolic blood pressure, and the level of hirsutism measured with the use of the

Ferriman Gallwey (FG) score. Weight and height were measured in light clothing without shoes. BMI was calculated as weight (kg) divided by height (m) squared. BMI values of ≥ 25 kg/m² were considered as overweight. BMI values of ≥ 30 kg/m² were deemed to be obese. Waist circumference was measured at the narrowest level between the costal margin and iliac crest, and the hip circumference was measured at the most comprehensive level over the buttocks while the subjects were standing and breathing normally. The waist-to-hip ratio (WHR) was calculated. A WHR >0.72 was considered abnormal (Ashwell et al., 1982). Clinical and biochemical hyperandrogenism was defined as an FG score $> eight$ and a free androgen index (FAI: $[(T/SHBG) \times 100] >4.5$). Subsequently, a systematic transvaginal or pelvic ultrasonography was performed to assess ovarian volume, endometrial thickness, and the total number of antral follicles measuring 2-10 mm. Finally, an extensive metabolic and endocrine profile was assessed.

2.1. Assessment of metabolic profile

Regarding the cardiovascular profile, BMI and systolic and diastolic blood pressure were evaluated in both PCOS and healthy women. In all women, waist circumference, fasting glucose, insulin, and lipid profile (i.e., total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol) were additionally measured. Insulin resistance was assessed by using the homeostasis model assessment (HOMA-IR: $[\text{fasting glucose (mmol/L)} \times \text{fasting insulin [mU/L]}]/22.5$) (Matthews et al., 1985). Metabolic syndrome was defined according to the National Cholesterol Education Program (NCEP) ADP III criteria (Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2002). According to this definition at least three of the following five features should be present (i) Waist circumference of > 88 cm (80 cm for Asians) (ii) Fasting blood glucose of ≥ 100 mg/dl (iii) Triglycerides ≥ 150 mg/dl (iv) HDL < 40 mg/dl (v) Blood pressure $> 130/85$ mm Hg. Thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, dehydroepiandrosterone sulfate (DHEAS), 17-hydroxyprogesterone (17-OHP), and total and free testosterone were checked in patients with PCOS and control subjects. Blood samples were collected at 8:30-9:30 a.m. after 12-h overnight fasting, in the early follicular phase (2nd-3rd days) after spontaneous or gestagen-induced menses in women. These parameters were quantified using a 7600-110 Automatic Analyzer (Hitachi Inc., Tokyo, Japan). The low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. Women with thyroid diseases, hyperprolactinemia, Cushing's disease, or congenital adrenal hyperplasia and those who were administered agents such as hormonal agents, ovulation-inducing agents, glucocorticoids, anti-androgens, or anti-hypertensives over the last three months prior to the study were excluded from the study.

2.2. Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences for Windows 22.0 software (SPSS, Chicago, IL., USA). Descriptive statistics were inferred as the mean, standard deviation, frequency, and percentage. Shapiro-Wilk test was utilized to check the normality of continuous variables. Parametric comparisons were performed using Student's t-test for normally distributed continuous data, and non-parametric comparisons were performed using the Mann-Whitney U test for non-normal continuous data distribution. Categorical variables were compared using chi-square or Fisher's exact tests, as appropriate. The Pearson Chi-square test was used to compare qualitative data. Spearman's Correlation Analysis was applied to evaluate the relationships between variables. A value of $p < 0.05$ was accepted as statistically significant.

3. Results

The demographic data of the PCOS and control groups are presented in Table 1. In this study, no statistically significant difference was found between patients with PCOS and control subjects in age, height, BMI, waist circumference, and waist-to-hip ratio ($p:0.428$, $p:0.995$, $p:0.073$, $p:0.313$, and $p:0.514$, respectively).

Table 1. Baseline characteristics of control group and PCOS women

	Control (n=91)	PCOS (n=109)	p
Age (years)	25.48±5.36	24.96±5.27	0.428
Weight(kg)	73.35±17.91	82.31±24.81	0.041
Height(cm)	160.01±6.70	160.06±6.19	0.995
BMI (kg/m ²)	28.89±7.76	32.27±10.27	0.073
WC (cm)	83.45±20.80	87.13±22.31	0.313
Waist/hip	0.78 ± 0.26	0.83 ± 0.26	0.514
SBP (mmHg)	111 ± 13	118 ± 13	0.013
DBP (mmHg)	71 ± 9	78 ± 12	0.028
FG score	5.26± 2.1	10.23± 2.14	0.004

BMI: body mass index; WC: waist circumference, SBP: systolic blood pressure; DBP: diastolic blood pressure. Results are expressed as median (range) or mean±SD according to distribution. $p < 0.05$, significant difference between groups

Weight, blood pressure (BP), systolic and diastolic, and also FG (Ferriman Gallway) scores were significantly higher in patients with PCOS compared to control subjects ($p:0.041$, $p:0.013$, $p:0.028$ and $p:0.004$, respectively, Table 1). In this study, no statistically significant difference was found between patients with PCOS and control subjects in FSH, estradiol, prolactin, TSH, fT₃, fT₄, and DHEASO₄ levels ($p:0.483$, $p:0.223$, $p:0.623$, $p:0.231$, $p:0.129$, $p:0.250$ and $p:0.274$ respectively). But, LH, cortisol, total and free testosterone, and 17-OH progesterone levels were significantly higher in patients with PCOS than control subjects ($p:0.000$, $p:0.003$, $p:0.005$, $p:0.003$ and $p:0.008$ respectively, Table 2). Also, in this study, vitamin B₁₂ and the 25-OH vitamin D₃ levels significantly lower in patients with PCOS compared to control

subjects ($p:0.048$ and $p:0.046$ respectively). HbA_{1C}, fasting blood glucose levels, fasting insulin levels and HOMA-IR were significantly higher in patients with PCOS compared to control subjects ($p:0.012$, $p:0.044$, $p:0.011$ and $p:0.006$ respectively, Table 2). Total-C, LDL-C, and TG levels were significantly higher in women with PCOS than control subjects ($p:0.041$, $p:0.035$, and $p:0.039$, respectively). Also, HDL-C levels were significantly lower in women with PCOS compared to control subjects ($p:0.034$).

Table 2. Hormonal and metabolic profile in the control group and PCOS women

	Control (n=91)	PCOS (n=109)	p
FSH (IU/L)	6.12 ± 1.32	5.98 ± 1.50	0.483
LH (IU/L)	7.35 ± 4.06	9.88 ± 4.89	0.000
Estradiol (pg/ml)	56.41±17.1	53.47±16.8	0.223
Prolactin (ng/ml)	16.39±7.23	16.12±7.31	0.623
TSH (mIU/L)	2.11±1.11	2.39±1.23	0.231
fT ₃ (mIU/L)	3.06±0.53	2.79±0.68	0.129
fT ₄ (mIU/L)	1.35±0.41	1.42±0.41	0.250
Cortisol (nmol/L)	14.29±6.68	16.81±7.36	0.003
TT (ng/dL)	37.56±35.0	61.20±28.2	0.005
fT (ng/dL)	1.57±0.43	2.32±1.37	0.003
DHEASO ₄ (nmol/L)	253.4±106.0	258.8±87.6	0.274
17-OH progesteron (ng/dl)	0.88±0.22	1.08±0.30	0.008
Vitamin B ₁₂ (pg/ml)	364.71±127.9	324.0±189.3	0.048
25-OH-D (nmol/L)	12.50±6.26	10.53±6.96	0.046
HbA _{1C}	5.09±1.32	5.44±1.36	0.012
Fasting glucose (mmol/L)	88.04±16.2	93.26±20.9	0.044
Fasting insulin (µIU/ml)	13.42±8.66	18.12±14.0	0.011
HOMA-IR	3.09±2.73	4.52±4.37	0.006
TC (mmol/L)	167.29±43.6	178.34±31.97	0.041
LDL-C (mmol/L)	92.48±29.59	99.28±26.3	0.035
HDL-C (mmol/L)	57.22±12.37	53.69±12.1	0.034
TG (mmol/L)	123.92±51.25	139.6±44.1	0.039

FSH: follicle stimulating hormone; LH: luteinizing hormone; FT₃: Free triiodothyronine; FT₄: Free thyroxine; TT: total testosterone; freeT: bioavailable testosterone; DHEASO₄: dehydroepiandrosteronesulphate; 25OHD: 25-hydroxyvitamin D; HOMA-IR: homeostatic model assessment of insulin resistance; TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol; TG: triglycerides; FAI: free androgen index; SHBG: sex hormone binding globulin; Results are expressed as median (range) or mean±SD according to distribution. $p < 0.05$, significant difference between groups

4. Discussion

This retrospective, cross-sectional study investigated whether the serum hormone, lipid, and vitamin levels were different in healthy women and in women with PCOS in the Black Sea region. Today most clinicians only focus on infertility in women with PCOS, and underestimate the risk of metabolic syndrome and other long-term effects. PCOS is not only a gynecologic condition affecting women of reproductive age, but also a part of metabolic syndrome with a variety of associated metabolic changes such as vitamin deficiencies, insulin resistance and dyslipidemia. Dokras et al. (2005) showed that 11-fold increase in the prevalence of metabolic

syndrome in PCOS patients compared with age-matched controls.

Chronic LH stimulation in PCOS reduces FSH receptor mRNA expression, which can induce the antral follicles to lose the FSH response (Orisaka et al., 2013). Decreased FSH reduces the expression of CYP19A1, which belongs to the cytochrome P450 family, by inactivating the cAMP-PKA-CREB pathway, thus leading to arrested follicular development (Gu et al., 2018). In the preovulatory follicular wave, acute and elevated insulin can decrease the percentage of large follicles that are ovulated, which reduces ovulatory oocyte fertilization. The effect becomes more obvious when insulin is combined with increased LH (Cadagan et al., 2016). Besides, the effect of insulin on follicle development may involve the insulin-like growth factor (IGF) system. Hyperinsulinemia can reduce IGF-binding proteins (IGFBP) synthesis to increase the free IGF-1 content (Stanek et al., 2007), which is the direct target of miR-323-3p (Wang et al., 2019). Decreased miRNA-323-3p can accelerate granulosa cell (GC) apoptosis via IGF-1 to inhibit folliculogenesis (Wang et al., 2019). Visceral adiposity a common occurrence in women with PCOS is strongly associated with insulin resistance and hyperlipidemia, both of which are harbingers of cardiovascular disease (Zheng and Li, 2016). Similar to the literature, in our study, body weights were heavier, and insulin resistance was found in the PCOS group. Many studies reported an increased risk for cardiovascular diseases in women with PCOS; however, the reason for this increased cardiovascular risk has not been clarified yet. PCOS is a chronic inflammatory disease, and an excess of visceral fat plays an important role in cardiovascular disorders. The inflammatory components of visceral obesity also affect atherosclerosis development (Mathieu et al., 2008). In our study, HbA1C, fasting blood glucose levels, fasting insulin levels, and HOMA-IR were significantly higher in patients with PCOS than in corresponding groups of healthy controls as in the literature (Legro et al., 1999; Hoffman and Ehrman, 2008). Insulin resistance and blunted carbohydrates oxidation were common features in all included women; thus, a common pathway of inappropriate energy oxidation, metabolic inflexibility, and response to insulin in women with PCOS is apparent (Rimmer et al., 2020). Insulin resistance is a risk factor for cardiovascular diseases, type II diabetes, hypertension, and dyslipidemia. Also, our results related to lipid profile in PCOS patients were compatible with the literature (Fulghesu et al., 2010). The most common lipid abnormalities among women with PCOS are elevated levels of triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), very-low-density lipoprotein cholesterol (VLDL-C), and low high-density lipoprotein cholesterol (HDL-C) levels (24). Similar to the literature, total-C, LDL-C, and TG levels were significantly higher in women with PCOS compared to control subjects in our study. Also, in our study, a low HDL-C level was found in the PCOS group. These lipid abnormalities are associated with insulin resistance and predict the development

of CVD, such as myocardial infarction (MI) (Wild et al., 2011). Obesity increases the risks of insulin resistance and cardiovascular disease and impairs reproductive functions (Bou et al., 2019). Obesity is commonly characterized by systemic and tissue-specific adipogenesis with increased cholesterol levels and lipid accumulation, which lead to inflammation, oxidative stress, and dysfunction in the ovaries (Araujo et al., 2018). Although obesity is the most critical marker of metabolic dysfunction, excess androgen levels contribute to increased metabolic syndrome risk independent of obesity (Rossi et al., 2008). In this regard, many studies have been conducted to investigate the close relationship between PCOS and metabolic syndrome. The mechanisms by which obesity is associated with dyslipidemia in women with PCOS include insulin resistance, overproduction of VLDL, abnormal lipoprotein lipase-mediated lipolysis, and a defect in the insulin-signaling pathway mediated by overexpression of PI3KR1 gene (Diamanti-Kandarakis et al., 2007). Testosterone induces dyslipidemia in women with PCOS through androgen receptor-mediated insulin resistance and the upregulation of genes responsible for the catabolism of HDL (Diamanti-Kandarakis et al., 2008). Clinical manifestations of MetS such as dyslipidemia develop from insulin resistance through increased secretion of non-esterified fatty acids and increased synthesis of TGs. In contrast, hypertension develops through endothelial damage and reduced nitric oxide bioavailability (Lim et al., 2019). Hypertension develops in women with PCOS from hyperaldosteronism via the activation of the renin-angiotensin system (Cascella et al., 2006). One cross-sectional study in Brazil of 233 women with PCOS and 70 controls found a higher prevalence of hypertension among the PCOS group (Marchesan et al., 2019) as in our study. Additionally, insulin resistance-related compensatory hyperinsulinemia has been implicated in the occurrence of hypertension in women with PCOS through an imbalance in the autonomic nervous system, increased renal sodium reabsorption, as well as a reduction in the production of nitric oxide (Marchesan et al., 2019). Although several studies have suggested that lower vitamin D levels are associated with an increased risk of insulin resistance and metabolic disturbance among women with PCOS the current findings are inconsistent (Van der Schueren et al., 2012). In our study, women with PCOS also had low vitamin D levels. The prevalence of Vitamin D deficiency (VDD) is 20%-48% among the general adult population, but a relatively higher prevalence of VDD is observed among women with PCOS, approximately 67%-85% of women with PCOS have VDD (Thompson et al., 2012). Several studies have confirmed an association between vitamin D status with cardiovascular diseases and diabetes-related outcomes (Camargo, 2011). Because vitamin D has anti-inflammatory effects, it is not surprising that it has beneficial effects on improving islet-cell functions, insulin release, and decreasing insulin resistance (Sung et al., 2012). PCOS presents alterations in metabolic pathways affected by Vitamin D, such as the insulin pathway, sex hormone production, and calcium

homeostasis (Li et al., 2011; Hassan et al., 2012). Patients with metabolic syndrome have low serum 25(OH)D levels, and restoration of serum 25(OH)D levels improves insulin resistance (Takiishi et al., 2012). One study in Tehran, however, did not find any difference in vitamin D levels in women with and without PCOS (Moini et al., 2015). In our study, serum vitamin B12 concentrations were significantly lower in PCOS women in comparison with control women as in the literature (Kaya et al., 2009). The low concentrations of vitamin B12 found in PCOS women with IR suggest the involvement of vitamin B12 in hyperinsulinemia, insulin resistance, and hyperhomocysteinemia. The importance of vitamin B12 in the remethylation of homocysteine to methionine is well recognized, and hyperhomocysteinemia is also a feature of vitamin B12 deficiency (Fonseca et al., 1999). The management of PCOS and metabolic syndrome includes lifestyle changes, especially for weight optimization, which has multiple clinical and personal benefits. Weight loss in overweight women could reduce the risk of suffering diabetes and death from cardiovascular causes by 28% (Feldman et al., 2017) and 21% (Look AHEAD Research Group, 2016), respectively. The low number of patients and retrospective study design were among the limitations of our study.

In this study, we found significant differences between patients with PCOS and control groups for hormone, lipid, and vitamin profiles. PCOS is an excess androgen disorder with different degrees of reproductive and metabolic dysfunctions. These effects are lifetime long, and lifestyle changes consisting of increased physical activity and caloric restriction can improve both metabolic and reproductive outcomes. As a result, effective diagnosis and treatment approaches are needed for protecting patients with PCOS from diabetes and cardiovascular diseases.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

References

- Abbot, D.H., Dumesic, D.A., Frank, S., 2002. Developmental origin of polycystic ovary syndrome—a hypothesis. *J. Endocrinol.* 174, 1-5.
- Araujo, J.F.P., Podratz, P.L., Sena, G.C., Merlo, E., Freitas-Lima, L.C., Ayub, J.G.M. et al., 2018. The obesogen tributyltin induces abnormal ovarian adipogenesis in adult female rats. *Toxicol. Lett.* 295, 99-114.
- Ashwell, M., Chinn, S., Stalley S., Garrow, J.S., 1982. Female fat distribution: a simple classification based on two circumference measurements. *Int. J. Obes.* 6, 143–152.
- Bou, N.L., Shi, H., Carr, B.R., Word, R.A., Bukulmez, O., 2019. Effect of Body Weight on Metabolic Hormones and Fatty Acid Metabolism in Follicular Fluid of Women Undergoing In Vitro Fertilization: A Pilot Study. *Reprod. Sci.* 26, 404-411.
- Cadagan, D., Khan, R, Amer, S., 2016. Thecal cell sensitivity to luteinizing hormone and insulin in polycystic ovarian syndrome. *Reprod. Biol.* 16, 53-60.
- Camargo, C.A., 2011. Vitamin D and cardiovascular disease: time for large randomized trials. *J. Am. Coll. Cardiol.* 58, 1442-1444.
- Cascella, T., Palomba, S., Tauchmanová, L., Manguso, F., Di Biase, S., Labella, D., et al., 2006. Serum aldosterone concentration and cardiovascular risk in women with polycystic ovarian syndrome. *J. Clin. Endocrinol. Metab.* 91, 395–400.
- Diamanti-Kandarakis, E., Papavassiliou, A.G., Kandarakis, S.A., Chrousos, G.P., 2007. Pathophysiology and types of dyslipidemia in PCOS. *Trends Endocrinol. Metab.* 18, 280–285.
- Dokras, A., Bochner, M., Hollinrake, E., Markham, S., VanVoorhis, B., Jagasia, D.H., 2005. Screening Women with Polycystic Ovary Syndrome for Metabolic Syndrome. *Obstet. Gynecol.* 106, 131–137.
- Dunaif, A., Segal, K.R., Futterweit, W., Dobrjansky, A., 1989. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes.* 38, 1165-1174.
- Feldman, AL., Griffin, S.J., Ahern, AL., Long, G.H., Weinehall, L., Fhärm, E. et al., 2017. Impact of weight maintenance and loss on diabetes risk and burden: a population-based study in 33, 184 participants. *BMC Public Health.* 17, 170.
- Fonseca, V., Guba, S.C., Fink, L.M., 1999. Hyperhomocysteinemia and the endocrine system: Implications for atherosclerosis and thrombosis. *Endocr. Rev.* 20, 738–759.
- Fulghesu, A., Magnini, R., Portoghese, E., 2010. Obesity-related lipid profile and altered insulin secretion in adolescents with polycystic ovary syndrome. *J. Adolesc. Health.* 46, 474–481.
- Gu, Y., Xu, W., Zhuang, B., Fu, W., 2018. Role of A-kinase anchoring protein 95 in the regulation of cytochrome P450 family 19 subfamily A member 1 (CYP19A1) in human ovarian granulosa cells. *Reprod. Fertil. Dev.* 30, 1128-1136.
- Hassan, N.E., El-Orabi, H.A., Eid, Y.M., Mohammed, N.R., 2012. Effect of 25-hydroxyvitamin D on metabolic parameters and insulin resistance in patients with polycystic ovarian syndrome. *Mid. East. Fertil. Soc. J.* 17, 176–180.
- Hoffman, L.K., Ehrmann, D.A., 2008. Cardiometabolic features of polycystic ovary syndrome. *Nat. Clin. Pract. Endocrinol. Metab.* 4, 215-222.
- Kaya, C., Dinçer, C.S., Satiroğlu, H., 2009. Obesity and insulin resistance associated with lower plasma vitamin B12 in PCOS. *Reprod. Biomed. Online.* 19, 721-726.
- Ketel, I.J., Serne, E.H., Ijzerman, R.G., Korsen, T.J.M., Twisk, J.W., Hompes, P.G.A. et al., 2011. Insulin-induced capillary recruitment is impaired in both lean and obese women with PCOS. *Hum. Reprod.* 26, 3130–3137.
- Legro, R.S., Kunselman, A.R., Dodson, W.C., Dunaif, A., 1999. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J. Clin. Endocrinol. Metab.* 84, 165-169.
- Li, H.W., Brereton, R.E., Anderson, R.A., Wallace, A.M., Ho, C.K., 2011. Vitamin D deficiency is common and associated with metabolic risk factors in patients with polycystic ovary syndrome. *Metabolism.* 60, 1475–1481.
- Lim, S.S., Kakoly, N.S., Tan, J.W.J., Fitzgerald, G., Bahri, K.M., Joham, A.E., et al., 2019. Metabolic syndrome in polycystic ovary syndrome: a systematic review, meta-analysis and meta-regression. *Obes. Rev.* 20, 339–352.
- Look AHEAD Research Group. Gregg, E., Jakicic, J., Blackburn, G., Bloomquist, P., Bray, G., Clark, J, et al., 2016. Association of the magnitude of weight loss and changes in physical fitness with long-term cardiovascular disease outcomes in overweight or obese

- people with type 2 diabetes: a post-hoc analysis of the Look AHEAD randomised clinical trial. *Lancet. Diabetes Endocrinol.* 4, 913–921.
23. Manousopoulou, A., Al-Daghri, N.M., Garbis, S.D., Chrousos, G.P., 2015. Vitamin D and cardiovascular risk among adults with obesity: a systematic review and meta-analysis. *Eur. J. Clin. Invest.* 45, 1113-1126.
 24. Marchesan, L.B., Spritzer, P.M., 2019. ACC/AHA 2017 definition of high blood pressure: implications for women with polycystic ovary syndrome. *Fertil. Steril.* 111, 579–587.
 25. Mathieu, P., Pibarot, P., Larose, E., 2008. Visceral obesity and the heart. *Int. J. Biochem. Cell Biol.* 40, 821–836.
 26. Matthews, D.R., Hosker, J.P., Rudenski, A.S., 1985. Homeostasis model assessment: insulin resistance and (cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 28, 412–419.
 27. McCarty, M.F., 2000. Increased homocysteine associated with smoking, chronic inflammation, and ageing may reflect acute phase induction of pyridoxal phosphatase activity. *Med. Hypotheses.* 55, 289–293.
 28. Moini, A., Shirzad, N., Ahmadzadeh, M., Hosseini, R., Hosseini, L., Sadatmahalleh, S.J., 2015. Comparison of 25-hydroxyvitamin D and calcium levels between polycystic ovarian syndrome and normal women. *Int. J. Fertil. Steril.* 9, 1–8.
 29. Orisaka, M., Hattori, K., Fukuda, S., Mizutani, T., Miyamoto, K., Sato, T., et al., 2013. Dysregulation of ovarian follicular development in female rat: LH decreases FSH sensitivity during preantral-early antral transition. *Endocrinology.* 154, 2870-2880.
 30. Poretsky, L., Piper, B., 1994. Insulin resistance, hypersecretion of LH, and a dual-defect hypothesis for the pathogenesis of polycystic ovary syndrome. *Obstet. Gynecol.* 84, 613-621.
 31. Rimmer, M., Tan, B.K., Teede, H., Thangaratinam, S., Bassel, H., 2020. Metabolic inflexibility in women with polycystic ovary syndrome: a systematic review. *Gynecol. Endocrinol.* 36, 501-507.
 32. Rossi, B., Sukalich, S., Droz, J., 2008. Prevalence of metabolic syndrome and related characteristics in obese adolescents with and without polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.* 93, 4780–4786.
 33. Rotterdam ESHRE/ASRM Sponsored PCOS Consensus Workshop Group (2004). Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil. Steril.* 81, 19–25.
 34. Schacter, M., Razieli, A., Friedler, S., 2003. Insulin resistance in patients with polycystic ovary syndrome is associated with elevated plasma homocysteine. *Hum. Reprod.* 18, 721–727.
 35. Stanek, M.B., Borman, S.M., Molskness, T.A., Larson, J.M., Stouffer, R.L., Patton, P.E., 2007. Insulin and insulin-like growth factor stimulation of vascular endothelial growth factor production by luteinized granulosa cells: comparison between polycystic ovarian syndrome (PCOS) and non-PCOS women, *J. Clin. Endocrinol. Metab.* 92, 2726-2733.
 36. Stein, I.F., Leventhal, M.L., 1935. Amenorrhea associated with bilateral polycystic ovaries. *Am. J. Obstet. Gynecol.* 29, 181-191.
 37. Sung, C.C., Liao, M.T., Lu, K.C., Wu, C.C., 2012. Role of vitamin D in insulin resistance. *J. Biomed. Biotechnol.* 634195.
 38. Takiishi, T., Gysemans, C., Bouillon, R., Mathieu, C., 2012. Vitamin D and diabetes. *Rheum. Dis. Clin. North. Am.* 38, 179-206.
 39. Thompson, R.L., Spedding, S., Buckley, J.D., 2012. Vitamin D in the aetiology and management of polycystic ovary syndrome. *Clin. Endocrinol.* 77, 343–350.
 40. Van der Schueren, B.J., Verstuyf, A., Mathieu, C., 2012. Straight from D-Heart: vitamin D status and cardiovascular disease. *Curr. Opin. Lipidol.* 23, 17-23.
 41. Wang, T., Liu, Y., Lv, M., Xing, O., Zhang, Z., He, X., et al., 2019. miR-323-3p regulates the steroidogenesis and cell apoptosis in polycystic ovary syndrome (PCOS) by targeting IGF-1. *Gene.* 683, 87-100.
 42. Wild, R.A., Rizzo, M., Clifton, S., Carmina, E., 2011. Lipid levels in polycystic ovary syndrome: systematic review and meta-analysis. *Fertil. Steril.* 95, 1073–1079.
 43. Zheng, S.H., Li, X.L., 2016. Visceral adiposity index as a predictor of clinical severity and therapeutic outcome of PCOS. *Gynecol. Endocrinol.* 32, 177–183.