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ORIGINAL ARTICLE

Circulating anti-Müllerian hormone levels in relation to nutritional status and selected adipokines levels in polycystic ovary syndrome

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Summary

Objective The aim of the study was to analyse the relationship between nutritional status, selected adipokines and plasma anti-Müllerian hormone (AMH) levels in women with polycystic ovary syndrome (PCOS).

Study Design Patients and Measurements A prospective, cross-sectional study, involving 87 PCOS (48 obese) women and 67 non-PCOS women (36 obese). Anthropometric parameters were measured, and body composition was determined by the bioimpedance method. Fasting serum glucose, androgens, FSH, LH, SHBG, insulin, AMH, apelin-36, adiponectin, leptin and omentin-1 were measured.

Results Plasma AMH levels were significantly higher in PCOS compared to the non-PCOS group (7·8 ± 4·3 ng/ml *vs* 4·4 ± 2·4 ng/ml; $P < 0{\cdot}001$). Furthermore, AMH levels were higher in both PCOS and non-PCOS normal weight than in obese subgroups (8·9 ± 4·4 ng/ml *vs* 7·0 ± 4·0 ng/ml; $P < 0{\cdot}05$ and 5·1 ± 2·4 ng/ml *vs* 3·9 ± 2·3 ng/ml; $P < 0{\cdot}05$). There were negative correlations between AMH levels and anthropometric parameters (body mass, BMI, fat mass and percentage, as well as waist circumference) and plasma omentin-1 concentrations ($R = -0{\cdot}28$, $P < 0{\cdot}001$; $R = -0{\cdot}30$, $P < 0{\cdot}001$; $R = -0{\cdot}36$, $P < 0{\cdot}001$; $R = -0{\cdot}34$, $P < 0{\cdot}001$; $R = -0{\cdot}23$, $P < 0{\cdot}01$; and $R = -0{\cdot}20$, $P < 0{\cdot}05$, respectively) in all study groups. In multiple regression analysis, circulating AMH level variability was explained by omentin-1 levels and anthropometric parameters (excluding waist circumference).

Conclusions In this observational study, nutritional status appears to be the main factor influencing circulating AMH levels independent of PCOS. The observed AMH association with omentin-1 levels suggests that this adipokine may be a link between hormonal dysfunction of adipose tissue related to obesity and decreased AMH secretion.

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Introduction

Anti-Müllerian hormone (AMH) produced by granulosa cells of primary, preantral and small antral follicles is recognized as a marker of ovarian reserve.[1–4] This hormone suppresses growing follicles by inhibition of follicle-stimulating hormone (FSH)-dependent aromatase expression and activation of luteinizing hormone (LH) receptors.[5]

AMH ovary secretion and plasma levels are age dependent. The highest AMH values are observed in pubertal age. They decrease slowly from the age of 25 years, are substantially lower in the premenopausal period, and not detectable after the menopause.[6–9] Furthermore, it was found that plasma AMH level is related to ovarian follicle count.[10] It was also observed that in polycystic ovaries the AMH production in granulosa cells is 75 times greater than in normal ovaries, and plasma AMH levels in PCOS women are 2–3 times higher than in healthy women.[8,9] Thus, it was suggested that AMH level is a marker of the severity of ovulatory disturbances in PCOS.[2]

Some previously published results indicate that nutritional status may influence circulating AMH levels. Lower plasma AMH levels were found in obese compared to normal weight non-PCOS women, and an inverse association between AMH levels and BMI values was shown.[11,12] This may reflect a decreased ovarian reserve and fertility in obese women along with a decreased response to gonadotropin stimulation during superovulation. It has also been suggested that obesity directly influences ovarian AMH synthesis.[13–17] Additionally, Su *et al.*[18] proposed that lower AMH levels in late reproductive age obese women are the effect of other physiologic processes independent on antral follicle count. In contrast,

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other studies revealed that nutritional status (defined according to WHO BMI criteria: underweight, normal weight, overweight and obesity) did not affect serum AMH levels in reproductive age[19] and premenopausal women.[20]

The contradictory results have also been obtained in studies that assessed the relationship between nutritional status and AMH level in PCOS women. Some authors have described lower AMH levels in obese PCOS women and an inverse association between AMH levels and BMI,[21–23] while other did not show relation between AMH and nutritional status in PCOS[24–28] or even positive correlation between AMH levels and BMI values.[29]

A recently published study has shown that leptin but not adiponectin may affect the AMH synthesis and signalling by the JAK2/STAT3 pathway in infertile women undergoing fresh IVF and ICSI cycles.[30] There seems to be an inverse association between insulin levels, HOMA-IR values, circulating RBP4 and AMH, as well as a positive correlation between AMH and adiponectin.[31] So far, there is a lack of studies that assess the relationships between circulating AMH and adipokines levels in PCOS. Our previously published studies revealed changes of circulating adiponectin, apelin, leptin and omentin-1 levels in PCOS.[32–34]

The aim of the study was to analyse the relationship between nutritional status, selected plasma adipokines and AMH levels in PCOS women.

Material and methods

We conducted a cross-sectional, prospective study involved 87 women with PCOS, (39 normal weight and 48 obese) with self-reported stable body mass during last 3-month period, referred from Endocrinological Gynaecology Outpatient Clinic located in Academic Centre after initial examination of menstrual disturbances with the suspension of PCOS to the Department of Endocrinological Gynaecology. The patients were recruited from 2010 to 2011. The short-term, routine in Poland, comprehensive, diagnostic hospitalization was not the part of the study. The day of admission was set after contact of the patients at the first day of menstruation to schedule the admission for the hormonal diagnostics on the 3–4 day of menstrual cycle. The diagnosis of PCOS was based on the Rotterdam ESHRE/ASRM criteria from 2003.[35] Only woman that possessed all three of the Rotterdam criteria were enrolled. Sixty-seven regularly menstruating women without clinical symptoms of hyperandrogenism (the control group) were recruited from the patients of Outpatient Metabolic Management Center diagnosed with obesity ($n = 36$) and from the normal weight patients ($n = 31$) of Endocrinological Gynaecology Outpatient Clinic referred for contraceptive advice. Patients suffering from Cushing's syndrome, thyroid dysfunctions, androgen-secreting tumour, enzyme deficiency (21-hydroxylase in particular), decreased ovary reserves, amenorrhoea and type 1 or type 2 diabetes were not enrolled. Any pharmacological therapy as well as smoking and alcohol abuse were among the exclusion criteria. The study was conducted after obtaining informed consent of each participant. The study protocol was approved by the Bioethical Committee of Medical University of Silesia.

Normal weight was defined as body mass index (BMI) between 18·5 to 24·9 kg/m^2 and obesity as ≥30·0 kg/m^2. The characteristics of the study groups are presented in Table 1.

Table 1. Patients characteristics'

	PCOS			Non-PCOS		
	All ($N = 87$)	Normal weight ($N = 39$)	Obese ($N = 44$)	All ($N = 67$)	Normal weight ($N = 31$)	Obese ($N = 36$)
Age (years)	25·4 ± 5·5	23·7 ± 4·5*‡‡	27·9 ± 5·8	25·7 ± 4·9	23·8 ± 4·3‖	27·3 ± 4·9
Body mass (kg)	80·0 ± 25·3	58·3 ± 8·0***‡‡‡	97·7 ± 20·2§§	76·6 ± 19·1	59·8 ± 7·1‖‖	91·1 ± 13·3
BMI (kg/m^2)	29·4 ± 8·8	21·3 ± 2·2***†‡‡‡	36·1 ± 6·3§§	28·3 ± 7·0	22·2 ± 2·0‖‖	33·4 ± 5·4
Body fat (kg)	32·8 ± 19·0	16·7 ± 5·1***†‡‡‡	45·9 ± 15·8§§	30·2 ± 14·3	18·5 ± 4·3‖‖	41·0 ± 11·4
Body fat (%)	38·0 ± 10·9	28·2 ± 5·5***†‡‡‡	46·0 ± 6·9§§	37·6 ± 8·5	30·7 ± 4·8‖‖	43·6 ± 6·0
Waist circumference (cm)	89·8 ± 18·7	73·0 ± 7·0***‡‡‡	103·7 ± 12·3§§	87·0 ± 18·0	71·0 ± 8·0‖‖	100·0 ± 10·0
Total cholesterol (mg/dl)	176·3 ± 34·0	167·7 ± 28·1	183·2 ± 37·0	173·6 ± 31·0	174·7 ± 31·4	177·5 ± 28·3
LDL- cholesterol (mg/dl)	106·6 ± 38·3	92·3 ± 31·2*	117·2 ± 42·7	99·5 ± 27·5	102·9 ± 29·1	105·8 ± 22·4
HDL- cholesterol (mg/dl)	45·7 ± 14·1%%	60·1 ± 16·3*†††‡	43·7 ± 13·1§§¶¶	57·7 ± 15·5	60·7 ± 16·2	55·6 ± 14·7
Triglycerides (mg/dl)	100·7 ± 55·2	67·0 ± 26·7***	121·5 ± 61·4§§¶	76·8 ± 30·5	70·9 ± 21·5‖	85·0 ± 31·4
Glucose (mmol/l)	5·2 ± 1·1%%	4·7 ± 0·5†	5·2 ± 0·7§§¶	4·7 ± 0·4	4·7 ± 0·6	4·8 ± 0·4
Insulin (μIU/ml)	12·6 ± 7·5%%	9·0 ± 4·5***	15·5 ± 8·3§§¶¶	8·0 ± 2·8	7·4 ± 2·3	8·5 ± 3·0
HOMA-IR	3·1 ± 2·7%%	2·0 ± 1·2***	4·0 ± 3·2§§¶¶	1·7 ± 0·6	1·5 ± 0·5	1·8 ± 0·6

*$P < 0·05$; **$P < 0·01$; ***$P < 0·001$ normal weight PCOS *vs* obese PCOS.
†$P < 0·05$; ††$P < 0·01$; †††$P < 0·001$ normal weight PCOS *vs* normal weight non-PCOS.
‡$P < 0·05$; ‡‡$P < 0·01$; ‡‡‡$P < 0·001$ normal weight PCOS *vs* obese non-PCOS.
§$P < 0·01$; §§$P < 0·001$ obese PCOS *vs* normal weight non-PCOS.
¶$P < 0·01$; ¶¶$P < 0·001$ obese PCOS *vs* obese non-PCOS.
‖$P < 0·01$; ‖‖$P < 0·001$; normal weight non-PCOS *vs* obese non-PCOS.
%$P < 0·01$; %%$P < 0·001$ all PCOS *vs* all non-PCOS.

Each study subject was examined within 3 and 5 days of her menstrual period. Anthropometric measurements (body mass and height) were performed, and BMI was calculated according to the standard formula. Body composition was assessed by the bioimpedance method using Bodystat 1500 (Douglas, Isle of Man). Venous blood samples (15 ml) were withdrawn in the mornings between 8·00 and 9·00 a.m., after an overnight fast (14 h) and collected according to recommendations of the kit manufacturers. Plasma and serum aliquots were frozen and stored at −70 °C.

Laboratory procedures

Plasma glucose and lipids were estimated by colorimetric methods using commercially available test kits (Roche Diagnostics GmBH, Mannheim, Germany). Serum insulin concentration was determined by enzyme-linked immunosorbent assay (ELISA) (DRG Instruments GmbH, Marburg, Germany) with a lower limit of sensitivity of 1·76 μIU/ml and intra- and interassay coefficients of variations of 2·2% and 4·4%, respectively. HOMA-IR index was calculated using the standard formula: HOMA-IR = fasting concentration of insulin (μIU/ml) × fasting concentration of glucose (mmol/l)/22·5.

Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), oestradiol (E_2), total testosterone, androstenedione, DHEA-S and SHBG were determined by ELISA (DRG Instruments GmbH) with a lower limit of sensitivity 0·86 mIU/ml, 1·27 mIU/ml, 0·35 ng/ml, 9·7 pg/ml, 0·083 ng/ml, 0·019 ng/ml, 0·044 μg/ml and 0·2 nmol/l, respectively; the respective intra- and interassay coefficients of variations were 5·5% and 6·1% for FSH, 5·6% and 6·2% for LH, 4·5% and 5·9% for PRL, 4·7% and 7·8% for E2, 3·6% and 7·1% for testosterone, 6·5% and 10·2% for androstenedione, 4·8% and 7·5% for DHEA-S and 5·3% and 9·0% for SHBG. The free androgen index (FAI) was calculated according to the standard formula.

The ELISA method was also used for measurements of plasma AMH (Immunotech, Prague, the Czech Republic), apelin-36 (Phoenix Pharmaceuticals, Burlingame, USA), leptin (TECOmedical AG, Sissach, Switzerland), adiponectin (TECOmedical AG) and omentin levels (DRG Instruments GmbH) with the lower limit of sensitivity of 0·08 ng/ml, 0·11 ng/ml, 0·2 ng/ml, 0·6 ng/ml and 0·5 ng/ml, respectively, intra- and interassay coefficients of variations were 4·6% and 4·6% for AMH, 5·0–10·0% and <15·0% for apelin-36; 6% and 7% for leptin; 5% and 6% for adiponectin; and 3·7% and 4·6% for omentin-1.

Statistic analysis

Statistical analysis was performed using STATISTICA 9.0 PL (StatSoft, Cracow, Poland) software and R software environment. There were no missing data in the database. The results are presented as mean values ± standard deviation. Distribution of variables was evaluated by the D'Agostino–Pearson test. Homogeneity of variances was assessed by the Levene test. Quantitative variables were compared with two-way ANOVA with Duncan test post hoc. The assessment of associations between variables was carried out with the multivariate skew t regression. Outliers were identified based on Cook's distance values. The Cook–Weisberg test was used to test the residuals for heteroskedasticity. Model calculation was performed, including evaluation of multicollinearity, which was assessed with the variance inflation factor (VIF). VIF should not be >5. Goodness of fit of obtained model was assessed with the F-test and determination coefficient R^2. All the results were considered as statistically significant with a P value of <0·05.

Results

The characteristics of the PCOS and non-PCOS subgroups, including metabolic parameters and hormonal profiles, are presented in Tables 1 and 2. Both obese subgroups were older than the normal weight subgroups (Table 1).

Plasma AMH levels were significantly higher in the PCOS than in the non-PCOS group. However, circulating concentrations of AMH in both PCOS and non-PCOS groups were significantly higher in normal weight than in obese subgroups. Moreover, plasma AMH levels were significantly higher in the normal weight PCOS subgroup than in obese PCOS, normal weight non-PCOS and obese non-PCOS subgroups (Table 2).

The plasma study adipokines levels are listed in Table 2.

Correlations between anthropometric parameters and AMH levels

There was an inverse correlation between AMH levels and body mass ($R = -0{\cdot}28$, $P < 0{\cdot}001$), BMI ($R = -0{\cdot}30$, $P < 0{\cdot}001$), fat mass and percentage ($R = -0{\cdot}36$, $P < 0{\cdot}001$ and $R = -0{\cdot}34$, $P < 0{\cdot}001$, respectively) and waist circumference ($R = -0{\cdot}23$, $P < 0{\cdot}01$). In addition, a negative correlation between AMH level and age was demonstrated ($R = -0{\cdot}29$, $P < 0{\cdot}001$).

Correlations between AMH and study adipokines

AMH levels correlated negatively with plasma concentrations of omentin-1 ($R = -0{\cdot}20$, $P < 0{\cdot}05$) – Fig. 1. There was no association between plasma AMH and adiponectin, apelin-36 and leptin levels.

Multiple regression analysis

In the multiple regression models, age, PCOS status, HOMA-IR values, plasma adiponectin, apelin-36, leptin and omentin-1 levels and anthropometric parameters (BMI, waist circumference, body fat mass and percentage, alternatively) were included as independent explanatory variables for plasma AMH levels in all study group (Table 3). Circulating AMH level variability was explained by adiponectin, omentin-1 levels and all anthropometric parameters (Table 3).

Discussion

The results of our cross-sectional study demonstrated that plasma AMH levels are associated with nutritional status in both

Table 2. Serum concentrations of hormones and plasma adipokines levels in analysed groups of PCOS and non-PCOS

	PCOS			Non-PCOS		
	All ($N = 83$)	Normal weight ($N = 39$)	Obese ($N = 48$)	All ($N = 67$)	Normal weight ($N = 31$)	Obese ($N = 36$)
FSH (mIU/ml)	6·2 ± 2·7	5·6 ± 1·6	6·8 ± 3·3	7·1 ± 8·5	5·3 ± 2·0	8·6 ± 11·3
LH (mIU/ml)	11·2 ± 6·7	9·6 ± 5·8*	12·6 ± 7·1§	10·3 ± 8·3	8·8 ± 4·8	11·6 ± 10·4
LH/FSH	1·9 ± 1·2	1·8 ± 0·9	2·1 ± 1·4	1·8 ± 1·3	1·8 ± 0·9	1·8 ± 1·6
PRL (ng/ml)	6·6 ± 3·4%	5·9 ± 3·0*†††‡	7·2 ± 3·7§	10·0 ± 6·9	10·7 ± 7·8	9·3 ± 6·2
Androstenedione (ng/ml)	2·4 ± 1·2	2·9 ± 1·1**†‡	2·1 ± 1·2	2·2 ± 1·1	2·3 ± 1·5	2·0 ± 1·1
DHEA-S (μg/ml)	2·9 ± 1·1	2·8 ± 1·0	2·9 ± 1·3	2·6 ± 1·3	2·9 ± 1·3	2·4 ± 1·3
Total testosterone (ng/ml)	0·8 ± 0·4	0·8 ± 0·5	0·7 ± 0·3	0·7 ± 0·9	0·6 ± 0·2	0·6 ± 0·3
Oestradiol (pg/ml)	52·1 ± 38·9	58·0 ± 46·4	47·3 ± 31·3	70·8 ± 60·5	65·8 ± 43·5	75·2 ± 72·4
SHBG (nmol/l)	29·6 ± 26·1%	40·1 ± 33·8***	21·1 ± 12·4§§¶¶	53·5 ± 86·5	73·3 ± 120·6‖	36·0 ± 28·2
FAI	3·8 ± 2·9%	3·2 ± 2·8*†	4·3 ± 3·0§§¶¶	2·5 ± 2·8	1·8 ± 1·5‖	3·0 ± 3·4
AMH (ng/ml)	7·8 ± 4·3%	8·9 ± 4·4*†††‡‡	7·0 ± 4·0§¶¶¶	4·4 ± 2·4	5·1 ± 2·4‖	3·9 ± 2·3
Apelin-36 (ng/ml)	2·1 ± 1·8	3·1 ± 2·2***	1·2 ± 0·7¶¶	2·3 ± 1·8	2·1 ± 2·2	2·4 ± 1·4
Adiponectin (μg/ml)	6·7 ± 3·8	8·4 ± 2·9***	5·3 ± 3·9¶	8·1 ± 4·9	9·9 ± 6·1‖	6·5 ± 2·8
Leptin (ng/ml)	55·7 ± 88·6%	16·9 ± 13·1***	87·3 ± 109·3¶¶¶	22·9 ± 16·4	14·0 ± 10·8‖‖‖	30·5 ± 16·7
Omentin (ng/ml)	252·5 ± 165·7%	218·7 ± 130·0†††‡‡	280·0 ± 186·7¶¶¶	523·3 ± 309·1	510·4 ± 308·0	533·1 ± 313·4

*$P < 0·05$; **$P < 0·01$; ***$P < 0·001$ normal weight PCOS *vs* obese PCOS.
†$P < 0·05$; ††$P < 0·001$ normal weight PCOS *vs* normal weight non-PCOS.
‡$P < 0·01$; ‡‡$P < 0·001$ normal weight PCOS *vs* obese non-PCOS.
§$P < 0·01$; §§$P < 0·001$ obese PCOS *vs* normal weight non-PCOS.
¶$P < 0·05$; ¶¶$P < 0·01$; ¶¶¶$P < 0·001$ obese PCOS *vs* obese non-PCOS.
‖$P < 0·05$; ‖‖$P < 0·01$; ‖‖‖$P < 0·001$ normal weight non-PCOS *vs* obese non-PCOS.
%$P < 0·001$ all PCOS *vs* all non-PCOS.

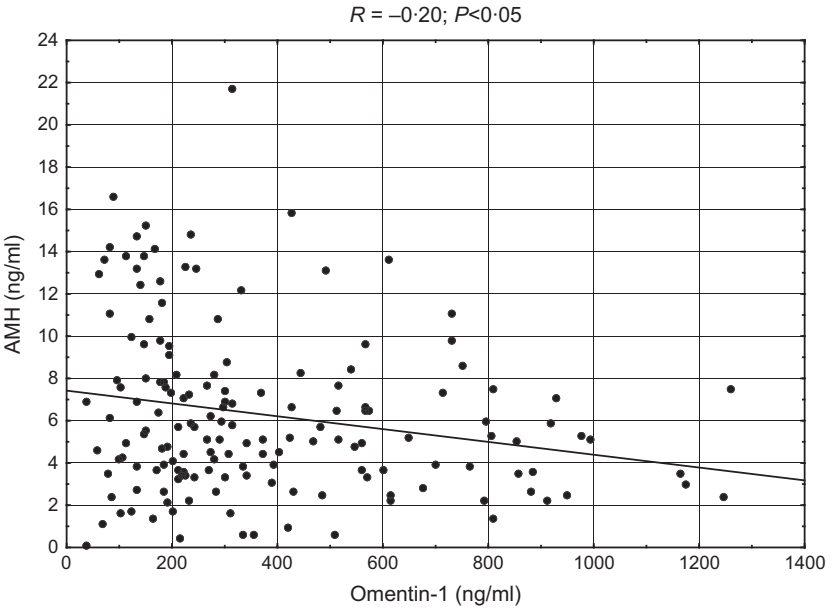

Fig. 1 Association between AMH and omentin-1 levels.

PCOS and non-PCOS women. Significantly lower values were observed in obese subgroups. In addition, an inverse relation between plasma AMH levels and body mass, BMI, fat mass and percentage as well waist circumference was found. The observed inverse association between plasma omentin-1 and AMH levels suggests that obesity-related hormonal dysfunction of adipose tissue is a factor leading to the reduction in ovarian reserve in both obese PCOS and non-PCOS women.

Table 3. Multivariate regression models examining the factors explaining AMH levels variability in the combined group of PCOS and non-PCOS women

	Model 1		Model 2		Model 3		Model 4		Model 5	
	β coefficient	*P*	β coefficient	*P*	β coefficient	*P*	β coefficient	*P*	β coefficient	*P*
PCOS	2·086	<0·001	2·276	<0·001	1·845	<0·001	1·933	<0·001	2·197	<0·001
Age	−0·145	<0·001	−0·118	<0·001	−0·118	<0·001	−0·095	<0·001	−0·132	<0·001
HOMA-IR	0·002	0·981	0·018	0·828	0·016	0·842	−0·009	0·899	−0·018	0·851
Adiponectin	−0·128	<0·01	−0·134	<0·001	−0·140	<0·001	−0·124	<0·001	−0·144	<0·001
Apelin-36	−0·198	0·173	−0·188	0·192	−0·288	<0·05	−0·287	<0·05	−0·172	0·226
Leptin	−0·002	0·310	−0·001	0·535	−0·002	0·249	−0·001	0·451	−0·002	0·343
Omentin-1	−0·002	<0·05	−0·002	<0·05	−0·002	<0·001	−0·002	<0·001	−0·002	<0·05
Body mass	−0·016	<0·05	–	–	–	–	–	–	–	–
BMI	–	–	−0·075	<0·05	–	–	–	–	–	–
Fat mass	–	–	–	–	−0·034	<0·001	–	–	–	–
Fat percentage	–	–	–	–	–	–	−0·062	<0·001	–	–
Waist circumference	–	–	–	–	–	–	–	–	−0·028	<0·05
Constants	6·862	<0·001	6·905	<0·001	6·421	<0·001	6·996	<0·001	7·817	<0·001

According to the results of previously published studies[22–25] circulating AMH levels were higher in PCOS than non-PCOS subgroups, regardless of nutritional status. The higher circulating AMH levels in PCOS are the result of increased production of this hormone by small follicles *per se* and increasing with their number.[25] On the other hand, AMH levels were lower in obese compared to normal weight subgroups independent of PCOS occurrence. This observation is further confirmed by the results of univariate correlation analysis and multivariate regression, showing an inverse relation between that plasma AMH levels and BMI, fat mass and its percentage, as well as waist circumference in all study groups. The results of previously published studies, in both PCOS and non-PCOS women, suggest that insulin resistance development related to obesity impairs granulosa cell function and declines of circulating AMH levels.[11,12,21,36,37] Contrary to these observations, we did not find any association between AMH levels and HOMA-IR values.

An experimental study showed that AMH expression, in both cumulus and mural granulosa cells, is suppressed by leptin treatment.[30] However, we did not observe the association between plasma leptin and AMH levels. Moreover, there was no association between AMH and apelin-36 levels. We found the association between circulating adiponectin and AMH levels but only in the multiple regression models, and the direction of this regression was inverse to previously reported by Park *et al.*[31] in non-PCOS women. Additionally, our study revealed an inverse correlation between circulating AMH and omentin-1 levels that was strengthened by the results of multiple regression analysis showing that circulating AMH level variability is explained by omentin-1 levels, independent of parameters describing nutritional status. The inconsistency seems to be the result of differences in adipose tissue hormonal function in PCOS and in non-PCOS women, as was described in our previously published studies.[32–34] We observed lower apelin-36 and adiponectin levels but higher leptin and omentin-1 levels in obese compared to normal weight PCOS women.[33,34] However, on the basis our recently published results, we cannot exclude that insulin resistance indirectly affect granulosa cell function by inhibition of omentin-1 synthesis in adipose tissue stromal cells.[34] It is also possible that disturbances of omentin-1 synthesis in adipose tissue are not the primary effect of visceral fat accumulation, but secondary to the development of insulin resistance and hyperandrogenism in PCOS.[34] It should be stressed that results of this study indicate that granulosa cells dysfunction in PCOS may be related to the impaired secretion of omentin-1 by stromal cells in women with severe hormonal disturbances of adipose tissue. Our previously published results shown that adiponectin-to-omentin-1 ratio is the good marker of adipose tissue dysfunction.[34] Thus, the lower AMH levels in obese PCOS women may be mainly related to endocrine dysfunction of stromal adipose tissue cells, while in non-PCOS obese women, the main role seems to play hormonal dysfunction of adipocytes.

It should be noted that our results do not elucidate the pathogenesis of PCOS but are another piece of evidence for the existence of a vicious cycle driving adipose tissue and ovarian hormonal disturbances and cannot explain the sequence of events. Only long-term observations of females, genetically predisposed to PCOS development, may explain the order of disturbances in the pathogenesis of PCOS.

The limitations of our study are size of the study subgroups and the lack of separation of PCOS with normal weight in subgroups with and without metabolic obesity. Additionally, the distribution of body fat and its visceral deposit were not directly assessed using DEXA or CT scan. Furthermore, insulin resistance assessment was performed using HOMA-IR, an indirect, however, widely accepted method. Moreover, in our study, only relationships between AMH and selected adipokines were analysed. We would also like to acknowledge that our study group included only the most severe phenotype of PCOS (phenotype A) assessed in hospital settings and thus made our cohort more

homogenous. However, the corresponding control group were recruited among the ambulatory patients.

Conclusions

In this observational study, nutritional status appears to be the main factor influencing circulating AMH levels independent of PCOS. The observed AMH association with omentin-1 levels suggests that this adipokine may be a link between hormonal dysfunction of adipose tissue related to obesity and decreased AMH secretion.

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Competing interests/financial disclosure

The Authors declare no conflict of interest or financial incentives.

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