Journal of Clinical & Translational Endocrinology 37 (2024) 100368

Contents lists available at ScienceDirect

Journal of Clinical & Translational Endocrinology

journal homepage: www.elsevier.com/locate/jcte

Hyperandrogenic eumenorrheic NON-PCOS women *versus* women with PCOS after the GnRH-agonist stimulation test preceded by suppression of adrenal steroidogenesis with dexamethasone

Salvatore Benvenga [a,b], Michele Russo [c], Gianpiero Forte [c], Vittorio Unfer [b,d,*]

[a] *Department of Clinical and Experimental Medicine, Section of Endocrinology, University of Messina, Messina, Italy*
[b] *The Experts Group on Inositol in Basic and Clinical Research and on PCOS (EGOI-PCOS), 00161 Rome, Italy*
[c] *R&D Department, Lo.Li Pharma, 00156 Rome, Italy*
[d] *UniCamillus-Saint Camillus International University of Health Sciences, 00156 Rome, Italy*

ARTICLE INFO

Keywords:
Hyperandrogenism
Hyperandrogenemia
Eumenorrhea
Polycystic ovary syndrome Gonadotropin-releasing hormone-agonist test
17-OH-progesterone

ABSTRACT

The subject of polycystic ovary syndrome (PCOS) has been extensively covered in the literature; however, there is a paucity of data regarding eumenorrheic women with hyperandrogenism and/or hyperandrogenemia without ultrasound evidence of PCO morphology (EuHyperA), and even less data on the comparison between PCOS and EuHyperA subjects. It has previously been shown that around half of PCOS women exhibit a hyper-response of serum 17-hydroxy-progesterone (17-OHP) to the stimulation by GnRH-agonists, also indicated as functional ovarian hyperandrogenism (FOH). Often, this stimulation test is preceded by suppression of the adrenal steroidogenesis with oral dexamethasone (Dex). FOH has been associated with an increase of the P450c17 activity in the ovaries driven by elevated insulin levels. Interestingly, treatment of women with PCOS with Dex suppression and GnRH-agonist stimulation (buserelin) highlighted the possible existence of two clusters of patients: hyper-responders (HR) and normal responders (NR).

In this retrospective study, we included 15 hyper-responders (HR) EuHyperA, 34 normal responders (NR) EuHyperA, 62 HR-PCOS and 45 NR-PCOS. The demographic characteristics, glucose-metabolism indices, and the hormonal response to Dex or buserelin were analyzed, with both intra-group and inter-group comparisons performed.

The rate of FOH was significantly greater in PCOS than EuHyperA women. Compared to HR-PCOS, HR-EuHyperA had [i.] significantly greater age at observation; [ii.] lower cortisol, 17-OHP, Δ4-androstenedione (Δ4-ASD), total testosterone (TT), LH, and buserelin-stimulated whole curve of dehydroepiandrosterone sulfate (DHEAS), 17-OHP, Δ4-ASD and TT. Compared to NR-PCOS, NR-EuHyperA had [i.] significantly greater FSH, and buserelin-stimulated whole curve of DHEAS; [ii.] significantly lower post-HD Dex Δ4-ASD, TT, buserelin-stimulated whole curve of 17-OHP, Δ4-ASD and TT. Compared to NR-PCOS, HR-PCOS had [i.] significantly greater body mass index (BMI), homeostasis model assessment for insulin resistance (HOMA-IR), cortisol, DHEAS, Δ4-ASD, TT, FT, FAI, E2, and insulin $AUC_{0\text{-}120min}$ (area under the curve) at oral glucose tolerance test (OGTT); [ii] higher levels of post-LD and post-HD Dex 17-OHP, Δ4-ASD, TT, post-HD Dex DHEAS (with greater levels indicating weaker adrenal suppression), whole curve of DHEAS, 17-OHP, Δ4-ASD, TT and LH; [iii] significantly lower sex-hormone binding globulin (SHBG).

Even if most of the parameters evaluated were statistically similar in the two sets of comparisons, interesting differences were observed. Women with PCOS exhibit higher androgen levels at baseline, after adrenal suppression and at the buserelin test, further to a higher ovarian volume. Of note, the percentage of women with HOMA-IR≥2.5 and serum insulin levels were greater in PCOS group compared to EuHyperA women. Moreover, within women with PCOS, the HR subgroup has higher insulin levels compared to the NR subgroup, when OGTT is performed. The alteration of the glucose-insulin balance and elevation of circulating androgens were more pronounced in PCOS, thus indicating that [i.] metabolic alterations might be crucial in the onset of PCOS itself and, [ii] EuHyperA might represent a milder form of PCOS.

* Corresponding author at: The Experts Group on Inositol in Basic and Clinical Research and on PCOS (EGOI-PCOS), 00161 Rome, Italy.
E-mail address: vunfer@gmail.com (V. Unfer).

https://doi.org/10.1016/j.jcte.2024.100368
Received 3 June 2024; Received in revised form 29 August 2024; Accepted 4 September 2024
Available online 6 September 2024

Introduction

An exaggerated response (also termed hyper-response) of 17-OHP to GnRH agonists was believed to reflect functional ovarian hyperandrogenism (FOH) as a result of an overactivation of the ovarian steroidogenic Δ4-pathway, and was considered to be a prominent abnormality in the majority of women with PCOS [1]. To confirm this hypothesis, Ehrman et al. [1] performed the GnRH agonist stimulation test (0.1 mg nafarelin, subcutaneously), under suppression of adrenal steroidogenesis by dexamethasone (Dex) in 40 women with hyperandrogenism who had oligomenorrhea, hirsutism (Ferriman-Gallwey score $\geq$ 8) or acne. FOH (that is, a serum 17-OHP peak $>$ 2.59 ng/ml) was detected in 23/40 subjects (57.5 %).

To date polycystic ovary syndrome (PCOS) has been already and deeply investigated in the literature; however, very little attention was given by researchers to eumenorrheic women with hyperandrogenism and/or hyperandrogenemia without ultrasound evidence of PCO morphology (EuHyperA), and even less to the comparison of the two conditions. In this regard, the purpose of our retrospective study was to compare these two groups of women, to assess their response to GnRH agonist stimulation test under suppression with Dex.

The first group (EuHyperA) consisted of women with (i.) eumenorrhea, (ii.) normal ovulation, (iii.) hyperandrogenism and/or hyperandrogenemia, and (iv.) no ultrasound evidence of polycystic ovaries. The second group comprised women with PCOS, diagnosed according to the Rotterdam criteria [2]. Clinical and biochemical comparisons and glucose metabolism-related parameters were evaluated and compared in addition to the frequency of FOH, the magnitude of elevation of steroid hormones and LH during the GnRH-agonist test, and the magnitude of steroid suppression following two sequential regimens of oral Dex (low dose and high dose). This double regimen permitted to maximize the differences observed in a similar study with an analogous setting by Pasquali et al. [3] (see Discussion). Comparative analysis was performed between the two groups (EuHyperA *vs* PCOS) and the two subgroups (defined as high responders [HR] and non-responders [NR] both intra-group (HR-EuHyperA *vs* NR-EuHyperA; HR-PCOS *vs* NR-PCOS) and inter-group (HR-EuHyperA *vs* HR-PCOS; NR-EuHyperA *vs* NR-PCOS). The rationale for this study was to verify whether the measured parameters were statistically similar between EuHyperA and PCOS, regardless of the specific 17-OHP response to GnRH-agonist admission, and whether EuHyperA may be considered a milder form of PCOS while establishing key differences between the two conditions.

Materials and methods

This is a retrospective study on consecutive women who attended the Endocrine Unit of the University Hospital of Messina between the years 1995 and 1999 for any of the following: hirsutism/acne/androgenic alopecia, oligo-/amenorrhea, ultrasonographic evidence of polycystic ovaries, detection of elevated serum levels of one or more androgens upon advice from their physician or own volition.

For the purposes of this study, we targeted two groups of women. The first group consisted of women with eumenorrhea, normal ovulation, and hyperandrogenism and/or hyperandrogenemia. This group is referred to as Eu-HyperA. The second group consisted of women with PCOS per Rotterdam criteria [2]. Since an important part of the study was to stratify both groups into NR or HR based on 17-OHP to the stimulation by a GnRH agonist, we selected women of either group based on the availability of 17-OHP measurements, 20 and 24 h after the treatment. As a result, 49 women were classified as Eu-HyperA women (34 NR and 15 HR) and 107 women with PCOS (45 NR and 62 HR). These patients were further specified, including patients who completed the two regimens of adrenal suppression before the GnRH agonist stimulation test and had a complete dataset for each tested parameter, resulting in a final patient pool of 41 Eu-HyperA women (28 NR and 13 HR) and 92 PCOS women (43 NR and 49 HR).

The age at first observation in the 156 women was 16 to 36 years, all patients were Caucasians, born and living in Sicily or Calabria.

Participants underwent a complete physical examination, including assessment of weight, height, body mass index (BMI), and Ferriman-Gallwey score. Eumenorrhea was defined as regular menses, namely menstrual cycles of 26 to 34 days. Normal ovulation was defined by serum progesterone levels $\geq$ 4 ng/ml between days 21 to 24 of the menstrual cycle. This progesterone level had to be confirmed in two subsequent menstrual cycles.

Hyperandrogenism was defined as either hirsutism, acne, and/or androgenic alopecia. Hirsutism was diagnosed according to a Ferriman-Gallwey score $\geq$ 8 [4]. Hyperandrogenemia was defined as the increased serum levels of any of TT, free testosterone (FT), free androgen index (FAI), Δ4-ASD, DHEAS. Patients exhibiting other causes of hyperandrogenism and hyperandrogenemia (*e.g.* nonclassical adrenal hyperplasia, Cushing' s syndrome, and androgen-secreting tumors) were excluded from the study.

Polycystic ovaries were identified following ultrasound assessment, with the presence of 12 or more follicles measuring 2 to 9 mm in diameter and/or at least one ovary having increased volume (>10 ml) [2].

Adrenal suppression and GnRH agonist testing

According to prior literature, the GnRH-analog testing was performed either with [1,3] or without [5–8] adrenal suppression of steroidogenesis, through the administration of oral Dex. To measure the effect of hormonal Dex-dependent suppression the following hormone levels were evaluated: cortisol, DHEAS, 17-OHP, Δ4-ASD, and TT.

Oral Dex was given as a low-dose regimen (2 mg/day) for two days, followed by a high dose regimen (8 mg/day) for subsequent two days. In detail, two blood samples were taken 30 min apart to serve as the pre-suppression baseline for hormone measurements, following this Dex administration began in the morning of the second or third day of the menstrual cycle. Dex was administered at a dose of 0.5 mg/day every 6 h for the first two days, until the 4th or 5th day of the menstrual cycle. At this point, following a further two blood samples which served as the post-low dose suppression hormone measurements, the Dex dose was increased to 2 mg every six hours for the next two days. Between 07:30 and 08:30 of the sixth or seventh day of the menstrual cycle, having taken 2 mg Dex, two blood samples were drawn 30 min apart to serve as the baseline for the GnRH-agonist test (−30 min and 0 min [also referred to as −0.5 h and 0 h]). For the purpose when measuring the post-high dose suppression, we used the lowest value for the hormonal parameters between −0.5 h and 24 h of the test, with virtually all values obtained 4 h post-injection. Immediately after the time 0 sampling, 0.5 mg of the GnRH agonist buserelin (Superfact®; Hoechst Marion Roussel SpA, Milano, Italy) was injected subcutaneously. Blood samples were taken at 1, 4, 20 and 24 h post-injection.

In 20 healthy women, we had preliminary established that the upper limit for a normal 17-OHP response was 2.49 ng/ml. Accordingly, peak values of 17-OHP equal to or above 2.50 ng/ml were considered abnormal response. Such peak values of 17-OHP typically occur at either at 20 h or 24 h time point.

Biochemical assays

Both in the eumenorrheic group and in the PCOS group, women with baseline 17-OHP levels $>$ 2.0 ng/ml underwent the $_{1-24}$ adrenocorticotropic hormone (ACTH) stimulation test (Synacthen, 250 μg) to exclude nonclassical adrenal hyperplasia due to 21-hydroxylase deficiency. Such deficiency was diagnosed if, 60 to 120 min after Synacthen injection, the 17-OHP peak was $>$ 10 ng/ml (>1000 ng/dl, >30.3 nmol/L) [9] which is a peak much lower than that (>100 ng/ml [>10,000 ng/dL or $>$ 300 nmol/L]) typical of the classic adrenal hyperplasia due to 21-hydroxylase deficiency [9].

In the amenorrheic women belonging to the PCOS group, human chorionic gonadotropin (hCG) was also measured at baseline to exclude pregnancy.

Insulin resistance was evaluated using HOMA-IR, calculated as follows: glycemia at 0 min [(mg/dl) × insulin at 0 min (μU/ml)]/405. OGTT (75 g) was performed in the morning between the 2nd and the 7th day of the menstrual cycle, taking blood samples at 0, 30, 60, 90 and 120 min for both glucose and insulin determination. Accordingly, we could calculate the $AUC_{0\text{-}120min}$ by the trapezoidal method for glycemia and insulinemia.

Serum FSH, LH, E2, TT, FT, Δ4-ASD, 17-OHP, DHEAS, cortisol, sex hormone-binding globulin (SHBG), and insulin were measured by immunofluorimetric ELISA or RIA assays. FAI was calculated using the formula TT (nmol/L) × 100/SHBG (nmol/L). All measurements of any given hormone (pre-Dex, post-Dex, pre-buserelin through 24-h post-buserelin) of any given woman were performed in the same run, as were glucose and insulin in the OGTT test. Intra-assay and inter-assay coefficients of variations of the biochemical indices measured were ≤ 5 % and ≤ 12 %, respectively.

Reference values in the early follicular phase are given in the footnote of Table 1.

Statistics

Hormone responses to the two Dex suppressions and buserelin stimulation were evaluated in terms of changes of (i.) absolute levels from the corresponding baseline levels and (ii.) percent changes from the corresponding baseline.

Data are reported as mean ± standard deviation (SD). Differences between means were evaluated by ANOVA or the Wilcoxon signed rank test, depending on the gaussian or non-gaussian distribution of the continuous variable. Differences between proportions were evaluated by the Fisher's exact test or chi-square (χ^2) test, as appropriate. P values of < 0.05 were considered statistically significant.

Results

Baseline data in the 49 EuHyperA and 107 PCOS women

The data from both groups are summarized in Table 1.

Compared to the women with PCOS, women with EuHyperA were approximately two-years older (p = 0.029), had a significantly smaller ovarian volume, in addition to significantly lower levels of 17-OHP and FT, and a 2-fold lower rate of 17-OHP hyper-response to buserelin. In contrast, the level of FSH was significantly higher in the EuHyperA group.

Baseline data stratified based on 17-OHP response to buserelin

As anticipated in the Introduction and Material and Methods, patients were stratified based on response to buserelin, resulting in the creation of two subgroups (HR and NR) within each study group (Table 2). Of note, it should be acknowledged that the size of the HR-PCOS subgroup is 4-times larger than the HR-EuHyperA, while the NR-PCOS subgroup is only 1.3-times larger than the NR-EuHyperA subgroup. Accordingly, one might anticipate that upon comparing the two subgroups within the EuHyperA group (n = 34 *vs* n = 15) there was lower statistical power than in the equivalent comparisons within the PCOS groups (n = 62 *vs* n = 45), and between the HR (n = 62 *vs* n = 34) and NR subgroups (n = 45 *vs* n = 15).

Between the EuHyperA HR and NR subgroups, there was no significant difference in age at presentation, Ferriman-Gallwey score, and ovarian volume, while DHEAS, FT, and SHBG were significantly different.

In contrast, compared with the NR-PCOS subgroup, the HR-PCOS had significantly greater BMI, HOMA-IR, 17-OHP, DHEAS, Δ_4-ASD, TT, FT, FAI, E2, LH and cortisol, and significantly lower SHBG. Of these 12 parameters, DHEAS, FT, and SHBG were also significantly different within the EuHyperA group. In contrast, HOMA-IR, HOMA-IR rate ≥ 2.5, 17-OHP, Δ4-ASD, TT, FAI, E2, LH and FSH were similar between the two EuHyperA subgroups.

Comparing the HR-PCOS with the HR-EuHyperA group, the first had significantly greater values of ovarian volume, serum levels of 17-OHP, Δ_4-ASD, TT, LH and cortisol, but significantly lower age at presentation.

The comparison between the NR-PCOS with the NR-EuHyperA group, demonstrated that the first had significantly greater ovarian volume and significantly lower FSH levels.

Table 1
Clinical and biochemical characteristics in the two study groups at first observation.

	EuHyperA	PCOS	P value
N	49	107	
Age	22.6 ± 4.6	20.8 ± 4.5	**p = 0.029**
Ferriman-Gallwey score	15.1 ± 5.0	14.9 ± 5.5	p = 0.81
Ovarian volume, ml	6.3 ± 2.4	10.6 ± 7.0	**p = 0.00026**
Body weight, Kg	63.8 ± 14.7	64.6 ± 12.3	p = 0.75
Body mass index (BMI), Kg/m^2	24.6 ± 5.6	24.6 ± 4.96	p = 0.98
HOMA-IR	2.09 ± 1.13	2.79 ± 1.67	p = 0.075
HOMA-IR≥2.5, rate	8/24 (33.3 %)	23/40 (57.5 %)	$X^2 = 3.51$, p = 0.061
17-OH-progesterone (17-OHP), ng/ml, baseline	0.92 ± 0.47	1.29 ± 0.64	**p = 0.00017**
Rate of 17-OHP hyper-response	15 (30.6 %)	62 (57.9 %)	$X^2 = 10.0$, **p = 0.0015**
Dehydroepiandrosterone sulfate (DHEAS), μg/ml	2.19 ± 1.18	2.22 ± 1.11	p = 0.75
Δ4-androstenedione (Δ4-ASD), ng/ml	2.62 ± 1.11	3.09 ± 1.42	p = 0.062
Total testosterone (TT), ng/ml	0.48 ± 0.20	0.56 ± 0.30	p = 0.42
Free testostosterone (FT), pg/ml	1.88 ± 1.51	2.58 ± 1.82	**p = 0.020**
Free Androgen Index (FAI)	4.7 ± 3.2	8.0 ± 10.8	p = 0.07
17-β-estradiol (E2), pg/ml	31.3 ± 21.0	28.1 ± 19.4	p = 0.39
Sex hormone-binding globulin (SHBG), nmol/L	45.6 ± 23.5	40.3 ± 18.8	p = 0.13
Luteinizing hormone (LH), mIU/L	4.51 ± 1.8	6.12 ± 4.2	p = 0.12
Follicle-stimulating hormone (FSH), mIU/L	6.47 ± 1.9	5.53 ± 1.9	**P=0.0048**
Serum cortisol, μg/dl	12.2 ± 3.6	12.9 ± 4.4	p = 0.37

Data of continuous variables are shown as mean ± SD.
P values in **bold** are statistically significant (**p < 0.05 or lower**).
Reference ranges for endocrine indices in the follicular phase were 2.3–10.3 mIU/L (FSH), 1.6–7.9 mIU/L (LH), 23–139 pg/ml (E2), 0.2–0.8 ng/ml (TT), 0–3.6 pg/ml (FT), 0.2–3.1 ng/ml (Δ4-ASD), 0.1–1.2 ng/ml (17-OHP), 1.2–3.6 μg/ml (DHEAS), 6.4–21.4 μg/dl (cortisol), 39–77 nmol/L (SHBG). FAI is normal when < 4.5.

Oral glucose-stimulation test (OGTT)

The patients who underwent the OGTT analysis are described in Table 3. Compared to the PCOS group, the EuHyperA group had significantly lower baseline glycemia.

Upon comparing the PCOS HR and NR subgroups, glycemia levels and glycemia $AUC_{0\text{-}120min}$ were similar (Fig. 1). However, the HR subgroup had significantly increased insulinemia levels at all time points of the OGTT and a statistically greater $AUC_{0\text{-}120min}$. In the equivalent

Table 2
Clinical and biochemical characteristics in the two study groups, each group being stratified based on their peak response (high [HR]] or normal [NR]) to the GnRH agonist buserelin.

	EHyperA			PCOS		
	HR, n = 15	**NR, n = 34**	**p value**	**HR, n = 62**	**NR, n = 45**	**p value**
Age	23.6 ± 4.5	22.1 ± 4.1	0.30	20.8 ± 4.1 [**p = 0.043**]	20.7 ± 4.9 [p = 0.19]	0.99
Ferriman-Gallwey score	15.6 ± 5.4	14.9 ± 4.9	0.53	14.0 ± 4.9 [p = 0.34]	15.6 ± 5.9 [p = 0.62]	0.24
Ovarian volume, ml	6.7 ± 2.8	6.11 ± 2.0	0.53	10.9 ± 6.1 [**p = 0.016**]	10.4 ± 7.8 [**p = 0.030**]	0.63
Body weight, Kg	70.3 ± 18.4	61.3 ± 12.5	0.062	67.2 ± 13.8 [p = 0.53]	62.2 ± 10.2 [p = 0.74]	0.089
BMI	26.7 ± 7.57	23.7 ± 4.2	0.093	25.8 ± 5.94 [p = 0.86]	23.5 ± 3.51 [P=0.83]	**0.041**
HOMA-index	2.78 ± 1.71	1.85 ± 1.0	0.28	3.47 ± 1.85 [p = 0.29]	2.33 ± 1.39 [p = 0.34]	**0.039**
HOMA-IR≥2.5, rate	3/7 (42.8 %)	5/17 (29.4 %)	0.34	12/16 (75.0 %) [p = 0.19]	11/24 (45.8 %) [p = 0.34]	0.10
17-OHP, ng/ml	0.97 ± 0.50	0.90 ± 0.46	0.57	1.52 ± 0.66 [**p = $3.8x10^{-5}$**]	0.97 ± 0.42 [p = 0.28]	**$1.3x10^{-9}$**
DHEAS, μg /ml	2.57 ± 1.21	2.03 ± 1.14	**0.011**	2.37 ± 1.24 [p = 0.42]	2.01 ± 0.87 [p = 0.96]	**0.020**
Δ4-ASD, ng/ml	2.44 ± 1.0	2.71 ± 1.15	0.38	3.32 ± 1.41 [**p = 0.0016**]	2.77 ± 1.37 [p = 0.94]	**0.0048**
TT, ng/ml	0.48 ± 0.22	0.48 ± 0.19	0.99	0.61 ± 0.28 [**p = 0.026**]	0.50 ± 0.33 [p = 0.89]	**0.0078**
FT, pg/ml	2.34 ± 1.18	1.67 ± 1.62	**0.023**	3.27 ± 1.82 [p = 0.065]	1.63 ± 1.35 [p = 0.94]	**$7.7x10^{-7}$**
FAI	5.3 ± 4.0	4.4 ± 2.7	0.73	10.5 ± 12.4 [p = 0.066]	5.3 ± 7.9 [p = 0.70]	**0.00017**
E2, pg/ml	24.3 ± 12.4	34.4 ± 23.3	0.29	30.3 ± 15.7 [p = 0.17]	25.2 ± 23.5 [p = 0.085]	**0.033**
SHBG, nmol/L	35.8 ± 15.9	50.0 ± 25.1	**0.033**	36.5 ± 19.1 [p = 0.90]	45.3 ± 17.3 [p = 0.33]	**0.0075**
LH, mIU/L	4.35 ± 1.60	4.40 ± 2.20	0.90	6.58 ± 3.91 [**p = 0.0023**]	5.49 ± 4.60 [p = 0.33]	**0.040**
FSH, mIU/L	6.09 ± 1.86	6.65 ± 1.91	0.34	5.78 ± 1.78 [p = 0.55]	5.17 ± 2.09 [**p = 0.0019**]	0.10
Serum cortisol, μg/dl	11.6 ± 3.5	12.5 ± 3.7	0.28	13.7 ± 4.7 [**p = 0.026**]	11.9 ± 3.8 [p = 0.32]	**0.0032**

Data for continuous variables are shown as mean ± SD.
The p values in brackets refer to the comparison between homologous groups, that is HR *vs.* HR and NR *vs.* NR.
P values typed **bold** are statistically significant (**p < 0.05 or lower**).
HR=High response to buserelin (17-OHP peak equal to or above 2.5 ng/ml), NR=Normal response to buserelin (17-OHP peak below 2.5 ng/ml). For other abbreviations, see Table 1.

analysis within the EuHyperA group, glycemia indices were similar to those within the PCOS group, with only the glycemia value measured at 120 min being significantly greater.

Compared to the NR subgroup, the HR-EuHyperA had significantly greater insulinemia at the last two time points and $AUC_{0\text{-}120\ min}$.

Comparing the NR-PCOS subgroup with its homologous NR-EuHyperA subgroup, the only appreciable difference concerned baseline glycemia, while no significant difference was observed between HR-PCOS and HR-EuHyperA.

Dex suppression

From here on, results refer to the 41 EuHyperA women (13 HR and 28 NR) and 92 PCOS women (49 HR and 43 NR) who completed the buserelin test under adrenal suppression with two sequential regimens of Dex (low dose [2 mg/d x 2 days] followed by high dose [8 mg/d x 2 days) (Table 4).

17-OHP was significantly different in the two study groups both pre Dex and after high dose Dex administration, with higher values in PCOS group. A significant difference was also observed for DHEAS and TT after high dose administration of Dex, in addition to higher values observed in the PCOS group compared to EuHyperA.

In terms of percent changes over pre-Dex levels (Fig. 2), the PCOS and EuHyperA groups significantly differ only in terms of TT post-high dose regimen (−11.2 %, p = 0.025).

Data (absolute levels) on Dex suppression stratified based on 17-OHP response to buserelin

Compared to NR PCOS, the HR PCOS group had significantly higher

Table 3
Basal glucose and insulin, and their corresponding responses to the oral glucose-stimulation test (OGTT) in the two groups of patients.

	EuHyperA	PCOS	p value
OGTT, n	24/49	40/107	
Glucose, 0 min [mg/dl]	80.1 ± 7.9	84.3 ± 7.5	**0.040**
30 min	117.7 ± 25.3	121.1 ± 28.7	0.64
60 min	126.2 ± 32.3	129.3 ± 36.6	0.74
90 min	110.9 ± 31.8	115.1 ± 24.4	0.55
120 min	98.7 ± 26.7	100.7 ± 20.3	0.73
AUC $_{0\text{-}120}$ min	13327 ± 2638	13787 ± 2487	0.49
Insulin, 0 min [μU/ml]	10.7 ± 6.0	13.7 ± 8.4	0.20
30 min	66.2 ± 41.6	92.1 ± 92.2	1.0
60 min	90.4 ± 53.8	124.1 ± 125.6	0.86
90 min	66.0 ± 36.7	96.1 ± 75.5	0.075
120 min	65.9 ± 44.4	86.6 ± 73.4	0.55
AUC $_{0\text{-}120}$ min	7830 ± 3963	10850 ± 9158	0.53

Data are shown as mean ± SD.
P values typed **bold** are statistically significant (**p < 0.05 or lower**).

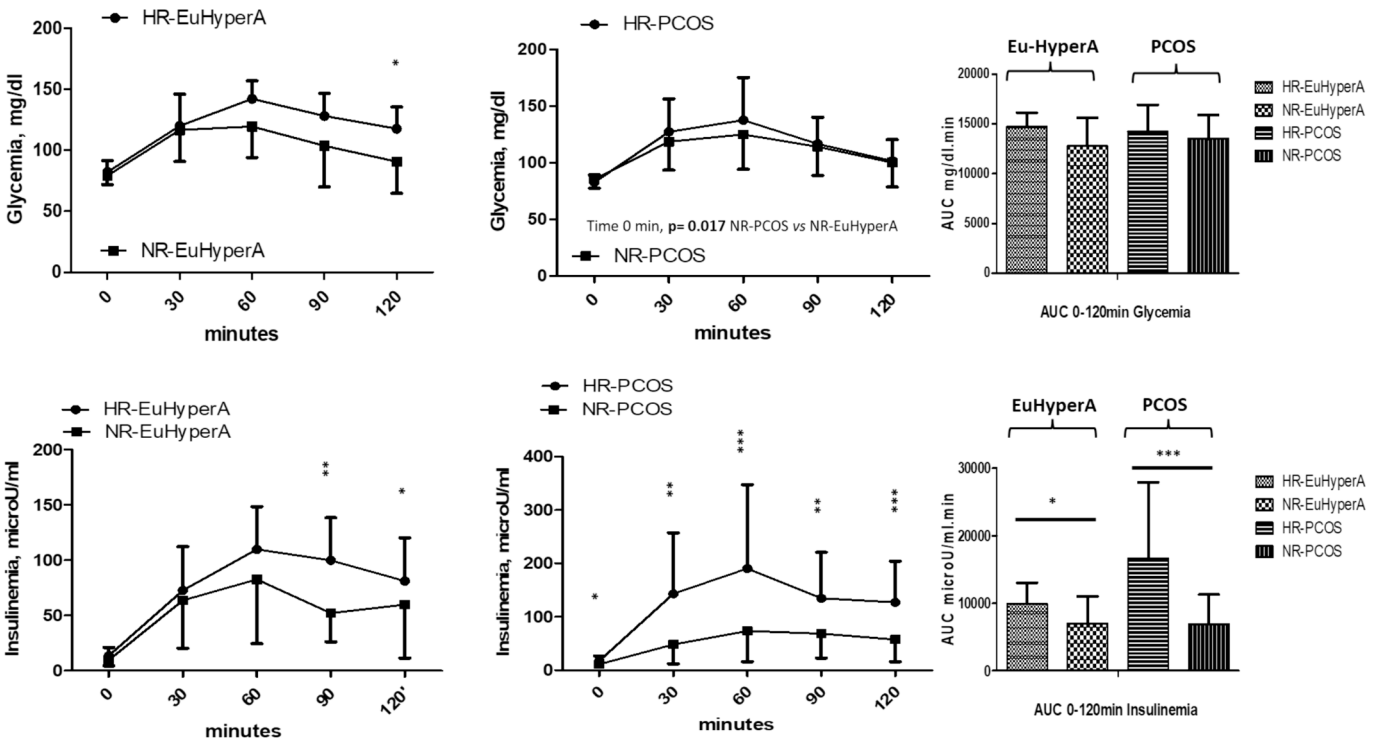

Fig. 1. Response of blood glucose (*top panels*) and insulin (*bottom panels*) to the oral glucose tolerance test (OGTT), also in terms of area under the curve (AUC), in the group of eumenorrheic women with hyperandrogenism/hyperandrogenemia (EuHyperA) and the group of women with polycystic ovary syndrome (PCOS), both groups being subdivided into two subgroups based on serum 17-OHP peak response to the buserelin test (hyper-response [HR; black circles] or normal response [NR; black squares]). Only comparisons that were statistically significant (p values: $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$). Data are mean ± SD.

TABLE 4
Serum steroid hormone levels prior to and after two sequential regimens of oral dexamethasone (Dex, low-dose [0.5 mg four times a day for the first two days] followed by high dose [2 mg four times a day for the subsequent two days]), with the buserelin test being performed in the morning of the 5th day soon after the last dose (2 mg) Dex.

Serum levels	EuHyperA (n = 41)	PCOS (n = 92)	p value
Cortisol, pre-Dex	12.2 ± 4.0	12.6 ± 4.7	0.72
, post low dose	0.39 ± 0.36	0.50 ± 0.49	0.49
, post high dose	0.46 ± 0.29	0.52 ± 0.80	0.67
DHEAS, pre-Dex	2.27 ± 1.26	2.18 ± 1.15	0.71
, post low dose	0.80 ± 0.48	0.71 ± 0.59	0.56
, post high dose	0.49 ± 0.31	0.49 ± 0.34	0.97
17-OHP, pre-Dex	0.94 ± 0.48	1.26 ± 0.66	**0.006**
, post low dose	0.29 ± 0.17	0.38 ± 0.25	0.14
, post high dose	0.25 ± 0.17	0.41 ± 0.31	**0.002**
Δ4-ASD, pre-Dex	2.63 ± 1.16	2.93 ± 1.35	0.23
, post low dose	0.93 ± 0.41	1.18 ± 0.69	0.091
, post high dose	0.59 ± 0.35	0.94 ± 0.70	**0.005**
TT, pre-Dex	0.47 ± 0.20	0.55 ± 0.31	0.13
, post low dose	0.11 ± 0.14	0.17 ± 0.17	0.14
, post high dose	0.06 ± 0.10	0.15 ± 0.16	**0.0009**

Values are shown as mean ± SD. P values typed **bold** are statistically significant (**p < 0.05 or lower**).

pre-Dex cortisol levels. However, the post-low dose and post-high dose cortisol levels were statistically similar. DHEAS levels were statistically greater only post-high dose in the HR-PCOS subgroup. After Dex, the Δ4-ASD levels were statistically greater in the HR subgroup compared with the NR subgroup both post-low and post-high dose. Moreover, 17-OHP and TT levels were statistically greater in HR-PCOS subgroup compared with the NR-PCOS subgroup before Dex, after low dose administration and post-high dose.

Post-Dex, 17-OHP and Δ4-ASD were statistically increased in the HR-EuHyperA group after both doses of Dex; furthermore, TT was also elevated following the low dose. The other differences were not statistically significant.

Comparing post-Dex hormone levels in the NR-PCOS subgroup *vs.* the NR-EuHyperA subgroup, statistically significant differences were observed for Δ4-ASD post-high dose and TT post-high dose, while comparing HR-PCOS with HR-EuHyperA the only significant differences were observed for 17-OHP and TT before Dex treatment.

Data (percent change) on Dex suppression stratified based on 17-OHP response to buserelin

Data for absolute levels shown in Table 5 are presented as percent decline from baseline in Fig. 3. Significant differences between the HR-PCOS subgroup and the NR-PCOS subgroup were observed in 17-OHP (after the high Dex dose), Δ4-ASD (after both doses) and TT (after the low dose).

Within the EuHyperA women, significant differences between the HR subgroup and the NR subgroup were observed in both 17-OHP and Δ4-ASD with the two Dex regimens, thus mimicking PCOS. The HR subgroup exhibited an overall lower degree of suppression both in PCOS and EuHyperA subgroups.

The buserelin test: Absolute levels and percent changes

Fig. 4 shows the time course of serum levels of each measured hormone in the EuHyperA group compared with its counterpart in the PCOS group.

The EuHyperA curve was significantly lower than the PCOS curve for

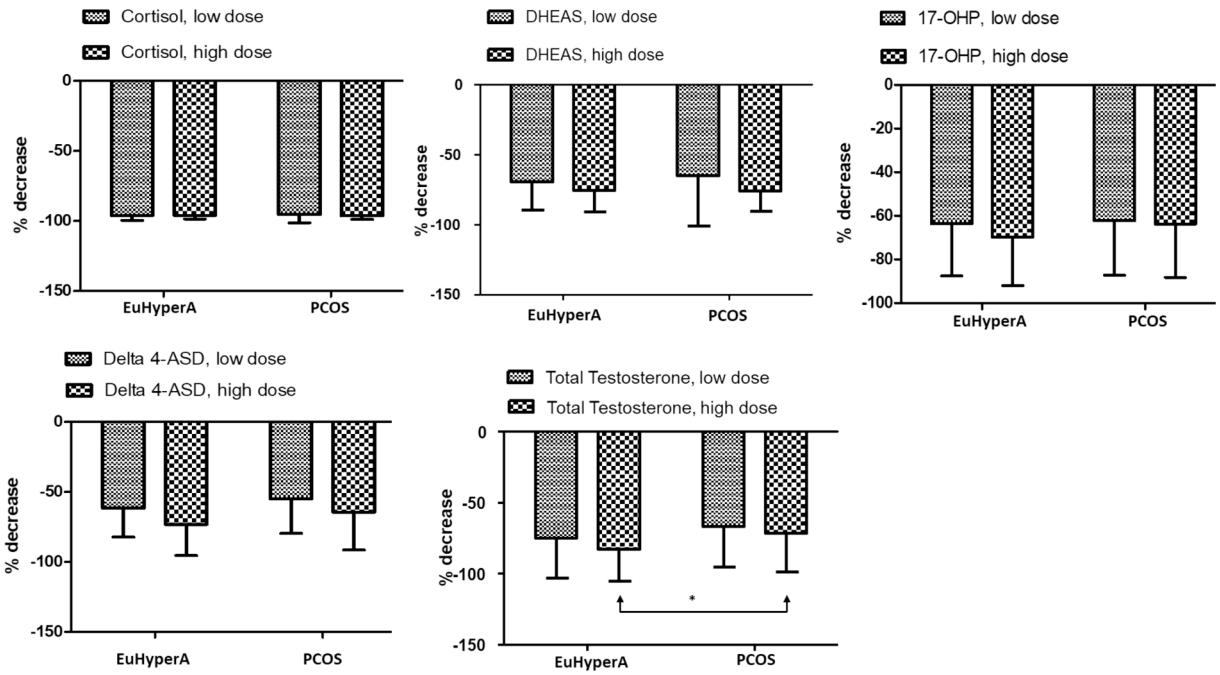

Fig. 2. Percent decrease (also referred to as % suppression) of serum steroid hormone levels after two sequential regimens of oral dexamethasone (low dose [0.5 mg four times a day for two days] followed by high dose [2 mg four times a day for the subsequent two days]). The buserelin test was performed in the morning of the fifth days, soon after the last 2 mg of dexamethasone. Only comparisons that were statistically significant (p values: $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$). Data are mean ± SD. In the subsequent Fig. 3, data of the two groups of patients are presented with stratification into two subgroups (HR and NR, see legend for Fig. 1).

17OHP, Δ4-ASD and TT; with significant difference recorded at all time points. For LH, the EuHyperA curve was not significantly lower than the PCOS curve; however, the two late time points were significantly lower. E2 levels were also significantly smaller in EuHyperA at 20 and 24 h. No difference was in DHEAS observed between the EuHyperA and PCOS groups.

In terms of percent increase of the 1–24 h segment of the curve, each of the tested hormonal parameters in the EuHyperA group were statistically similar to the corresponding hormone in the PCOS group.

The buserelin test: Absolute levels and % changes in women stratified based on 17-OHP response

As shown in Fig. 5, in the HR-EuHyperA *vs* NR-EuHyperA comparison of whole curves, curves of all hormones except DHEAS and E2 were significantly different, the first subgroup having higher levels. In the equivalent comparison within the PCOS group, the HR-PCOS group demonstrated significantly increased levels of all measured hormonal parameters except for E2 in comparison to the NR-PCOS group.

Concerning E2, its serum levels were greater at the two later time points (20 h and 24 h) in the HR subgroup compared to the NR subgroup in both PCOS and EuHyperA groups, but only significantly so within the PCOS group.

Upon comparing the two HR subgroups, the PCOS group had significantly greater curves of DHEAS, 17-OHP, Δ4-ASD and TT, while E2 and LH were not significantly increased.

Upon comparing the two NR subgroups, the same pattern described for the two HR subgroups was observed. The only exception was DHEAS, since the whole curve of NR-PCOS was significantly lower than that of the NR-EuHyperA.

The percent increase of DHEAS in the HR subgroup was significantly increased over the NR subgroup (41.8 ± 59.6 *vs*. 27.4 ± 75.4 %); the corresponding values for LH were 720 ± 571 *vs*. 1139 ± 1302 %. In the equivalent comparison of PCOS women, the only significant result was LH (975 ± 946 *vs*. 790 ± 827, p = 0.045).

Discussion

Herein we have demonstrated that while EuHyperA and PCOS women share various characteristics, the two groups differ significantly in several key parameters, which may hint at a different pathophysiology of the two conditions.

When the PCOS and EuHyperA groups of were compared at baseline, we observed that body weight, BMI and hirsutism score overlap in the two groups, while serum androgens, particularly DHEAS and FT, were higher in women with PCOS. Of note, FT is significantly greater likely due to the lower SHBG levels of PCOS group. It is important to highlight that hyperandrogenism was pronounced in PCOS group despite only three out of four PCOS phenotypes being characterized by hyperandrogenism according to the Rotterdam criteria [10]. Distinction of the hyper-androgenic phenotypes from the normo-androgenic phenotype D, is of increasing importance as evidence suggests that these women may represent different clinical pictures with divergent etiopathogenesis [11].

Androgen production in the ovary of women with PCOS is frequently associated with an increased ovarian volume [12]. In accordance with prior data, we observed a significantly higher ovarian volume in PCOS compared to EuHyperA at baseline. Androgen production takes place in the theca cells of the ovarian stroma, with the hyper-activity of these cells in the stromal area of the ovary [13–15] contributing to the increase of ovarian volume [16] as also found in the present study.

Of note, within this study serum levels of 17-OHP were significantly more elevated in women with PCOS, as a bench mark of an increased FOH, which was originally described to be typical of women with PCOS [1], though it also occurs in EuHyperA women but with a lower frequency. In our series of patients, FOH occurred in 58 % of women with PCOS compared to 31 % of the EuHyperA group.

As mentioned earlier in the text, hyper-response of 17-OHP was believed to be reflective of FOH resulting from an overactivation of the steroidogenic Δ4-pathway within the ovaries and was considered a prominent abnormality of PCOS [1]. However, in this study only 107

Table 5
Serum steroid hormone levels prior to and after two sequential regimens of oral dexamethasone (low dose [0.5 mg four times a day for the first two days] followed by high dose [2 mg four times a day for the subsequent two days]), with the buserelin test being performed in the morning of the 5th day soon after the last dose (2 mg) of dexamethasone. Each group of women is stratified based on the 17-OHP peak response to buserelin.

	EuHyperA			PCOS		
	HR (n = 13)	**NR (n = 28)**	**p value**	**HR (n = 49)**	**NR (n = 43)**	**p value**
Cortisol, pre-Dex	11.0 ± 3.7	12.8 ± 4.0	0.17	13.6 ± 5.0 [p = 0.082]	11.3 ± 4.1 [p = 0.13]	**0.026**
, post low dose	0.47 ± 0.48	0.32 ± 0.21	0.64	0.57 ± 0.50 [p = 0.62]	0.44 ± 0.47 [p = 0.74]	0.41
, post high dose	0.51 ± 0.43	0.44 ± 0.22	0.86	0.44 ± 0.29 [p = 0.51]	0.62 ± 1.14 [p = 0.66]	0.99
DHEAS, pre-Dex	2.69 ± 1.28	2.07 ± 1.23	0.14	2.30 ± 1.26 [p = 0.32]	2.05 ± 1.0 [p = 0.94]	0.33
, post low dose	0.76 ± 0.58	0.82 ± 0.42	0.77	0.77 ± 0.54 [p = 0.95]	0.66 ± 0.62 [P=0.086]	0.20
, post high dose	0.44 ± 0.28	0.51 ± 0.32	0.51	0.56 ± 0.39 [p = 0.33]	0.41 ± 0.26 [p = 0.14]	**0.043**
17-OHP, pre-Dex	0.93 ± 0.47	0.95 ± 0.50	0.92	1.53 ± 0.72 [**p = 0.0067**]	0.95 ± 0.40 [p = 0.99]	**2.2 x10^{-5}**
, post low dose	0.37 ± 0.16	0.23 ± 0.16	**0.042**	0.48 ± 0.29 [p = 0.25]	0.29 ± 0.18 [p = 0.36]	**0.0041**
, post high dose	0.38 ± 0.19	0.19 ± 0.16	**0.0027**	0.54 ± 0.34 [p = 0.14]	0.27 ± 0.19 [p = 0.06]	**2.8 x10^{-5}**
Δ4-ASD, pre-Dex	2.30 ± 1.05	2.78 ± 1.19	0.22	3.05 ± 1.18 [**p = 0.044**]	2.78 ± 1.53 [p = 0.99]	0.37
, post low dose	1.14 ± 0.46	0.78 ± 0.30	**0.035**	1.44 ± 0.64 [p = 0.21]	0.96 ± 0.66 [p = 0.34]	**0.0093**
, post high dose	0.85 ± 0.41	0.47 ± 0.25	**0.0008**	1.13 ± 0.81 [p = 0.23]	0.72 ± 0.46 [**p = 0.0086**]	**0.0071**
TT, pre-Dex	0.45 ± 0.20	0.48 ± 0.21	0.72	0.63 ± 0.27 [**p = 0.035**]	0.47 ± 0.34 [p = 0.87]	**0.018**
, post low dose	0.19 ± 0.17	0.05 ± 0.09	**0.031**	0.22 ± 0.17 [p = 0.65]	0.13 ± 0.17 [p = 0.11]	**0.033**
, post high dose	0.10 ± 0.13	0.04 ± 0.08	0.18	0.19 ± 0.18 [p = 0.081]	0.10 ± 0.12 [**p = 0.03**]	**0.02**

Values are shown as mean ± SD.
P values in brackets refer to the comparison between homologous groups, that is HR *vs* HR and NR *vs* NR. P values typed **bold** are statistically significant (**p < 0.05 or lower**).

women with PCOS (58 %) demonstrated FOH, a number which is in agreement with prior literature. Thus, as underscored in an Italian study on 148 PCOS per NIH criteria (rate of FOH=47 %) [3], FOH cannot be considered a reliable hallmark of PCOS since it is absent in approximately half of PCOS cases.

We found that the presence of FOH, and its association with HR status, accounts for some of the differences observed between the EuHyperA and PCOS groups, in addition to the individual HR and NR subgroups of each study group.

Indeed, following buserelin test, Each subgroup of PCOS patients had a higher ovarian volume compared to its EuHyperA counterpart. Interestingly, the HR-PCOS exhibited greater levels of 17-OHP, Δ4-ASD, and TT compared to HR-EuHyperA. Furthermore, when compared to NR-PCOS, HR-PCOS had higher levels of androgens in general in addition to FAI. In the aggregate, these data indicate that hyperandrogenemia is associated with a more pronounced FOH and 17-OHP levels, a trend that is maintained in both PCOS and EuHyper.

Beside the endocrinological pattern described, interesting aspects can be observed in the metabolic evaluation in the study population. In detail, after GnRH-agonist administration, BMI and HOMA-IR were significantly increased in HR-PCOS with respect to NR-PCOS. This difference was confirmed after OGTT, demonstrating further significant differences between the HR subgroup of PCOS. Indeed, insulinemia was higher in HR-PCOS compared to the NR-PCOS group, with an overlapping trend also being observed in HR- EuHyperA with respect to NR-EuHyperA.

These data suggest a difference in the metabolic status of the two study groups. Specifically, women with PCOS exhibit higher insulin levels compared to EuHyperA, thus raising the possibility of distinguishing between the two groups based upon metabolic status. Typically, PCOS is a condition closely associated with metabolic alterations, with women with PCOS exhibiting an increased risk of developing metabolic syndrome, increased BMI, obesity, and type 2 diabetes, compared to healthy controls [17–19]. Furthermore, it is estimated that about 75 % of women affected by PCOS are also affected by insulin-resistance [20]. As recently discussed [21], the insulin resistance-associated hyperinsulinemia may lead to hyperandrogenism/hyperandrogenemia, suggesting that the hyperandrogenic phenotypes of PCOS may be the consequence of a metabolic onset, which triggers the ovarian manifestations [11,22]. It can thus be hypothesized that the presence of metabolic alterations in the PCOS group are responsible for the increased androgen production observed in these patients in comparison to the EuHyperA group, the latter group demonstrating fewer metabolic unbalances. Even if the metabolic alterations are not currently considered a diagnostic feature of PCOS according to the international clinical guidelines [10], the importance of screening for metabolic interactions is regularly recommended [23].

Interestingly, insulin resistance, may affect the ovaries of PCOS patients, and exerts a role in FOH. Specifically, it is known that insulin sensitizes ovaries to LH by promoting the 17,20-lyase activity of

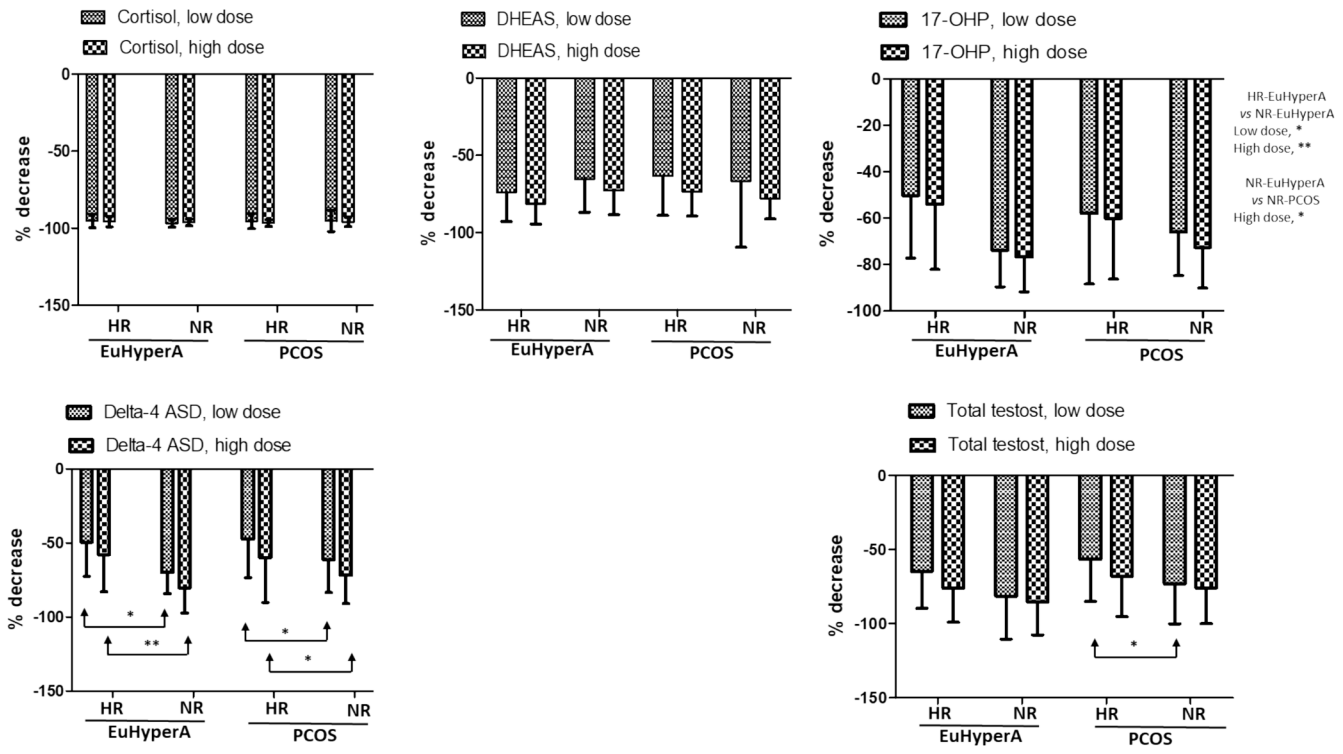

Fig. 3. Percent decrease (also referred to as % suppression) of serum steroid hormone levels after two sequential regimens of oral dexamethasone (see legend for Fig. 2) in the two groups of patients (EuHyperA and PCOS), both groups being subdivided into two subgroups (HR and NR [see legend for Fig. 1]). Only comparisons that were statistically significant (p values: $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***). Data are mean ± SD.

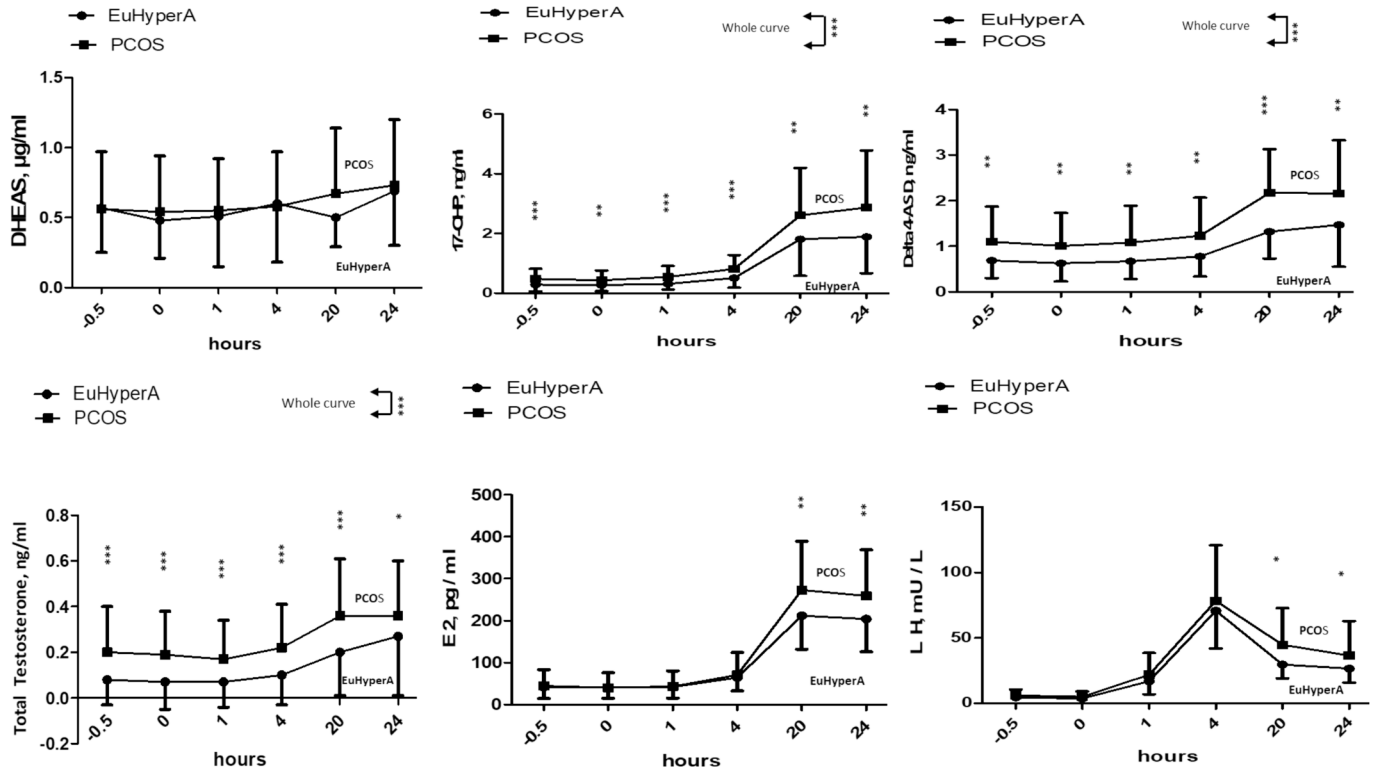

Fig. 4. Hormone responses to the buserelin test in the two groups of patients (EuHyperA and PCOS). Except for the absolute values of the whole curves and the percent increase of the 1 h-to-24 h curve over baseline, only comparisons that were statistically significant (p values: $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***). Data are mean ± SD. Due to the extremely irregular intervals of time points (from as little as 30 min of the first two [-0.5 h and 0 h] to as much as 16 h between 4 h and 20 h, in order not to use breaks in the time scale and not to squeeze the graphs, time points are represented equally spaced.

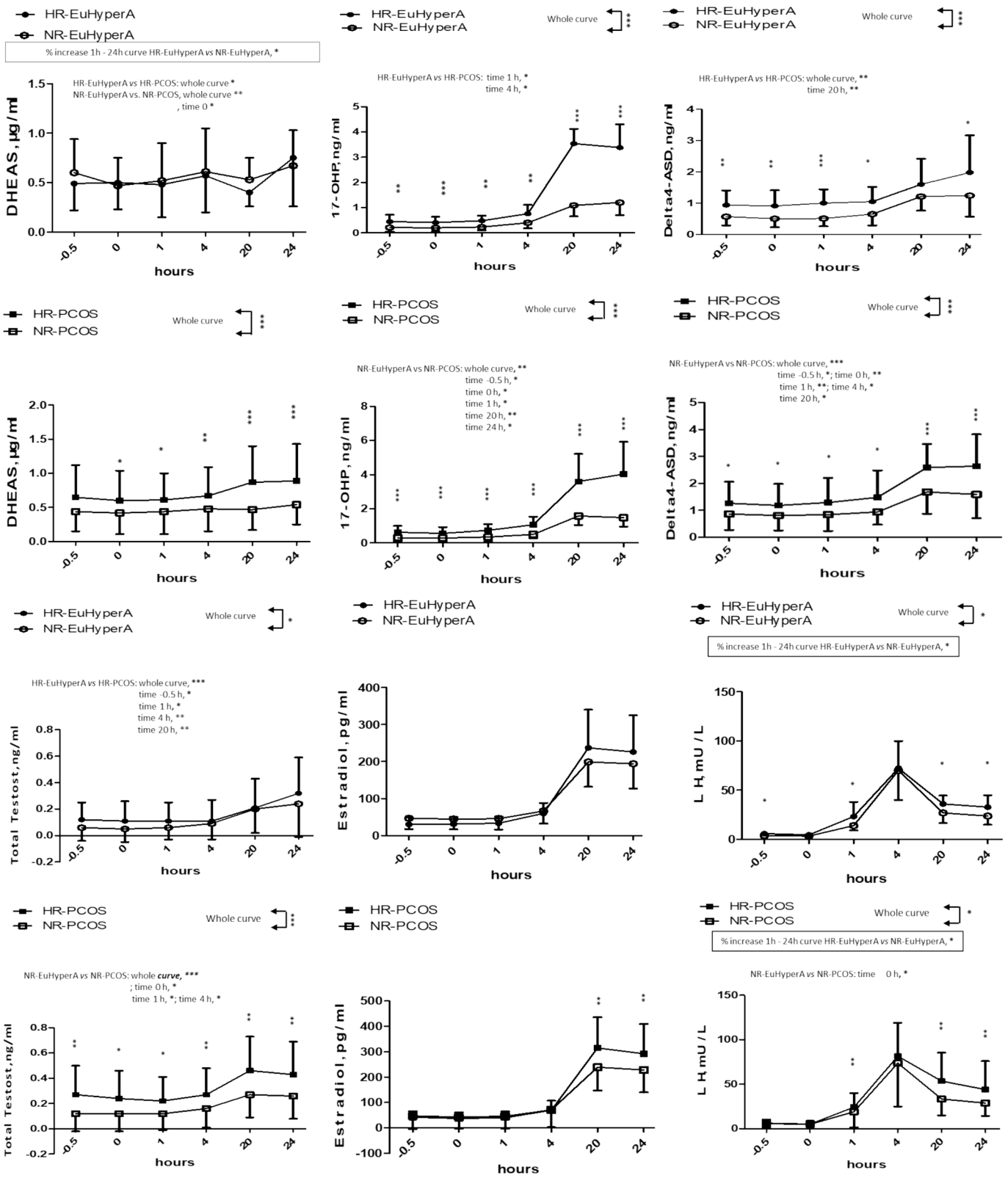

Fig. 5. Hormone responses to the buserelin test in the two groups of patients (EuHyperA and PCOS), both groups being subdivided into two subgroups (HR and NR [see legend for Fig. 1]). Except for the absolute values of the whole curves and the percent increase of the 1 h-to-24 h curve over baseline, only comparisons that were statistically significant (p values: $p < 0.05 = *$, $p < 0.01 = **$, $p < 0.001 = ***$). Data are mean ± SD. Due to the extremely irregular intervals of time points (from as little as 30 min of the first two [-0.5 h and 0 h] to as much as 16 h between 4 h and 20 h, in order not to use breaks in the time scale and not to squeeze the graphs, time points are represented equally spaced.

P450c17 [24]. Other interesting facts emerged from the analysis of the results associated with both the buserelin test and the adrenal suppression with two doses of Dex that precedes the buserelin test. In the GnRH-agonist test, the significantly higher curves of 17-OHP, Δ4-ASD and TT in the PCOS group compared to the EuHyperA were accounted for by the corresponding HR subgroups and NR subgroups; therefore, serum levels of 17-OHP, Δ4-ASD and TT are greater in PCOS women prior to the GnRH agonist injection and the trend is confirmed throughout the 24 h post-injection.

The buserelin-stimulated LH secretion resulted in LH curves that in the PCOS group were significantly higher compared to the EuHyperA group, particularly in the HR sub-groups. Also, LH levels in HR-PCOS were significantly higher compared to NR-PCOS. Moreover, a significant difference in FSH was observed at entry visit, with lower values observed in patients with PCOS. This difference is not maintained following buserelin treatment, as despite lower FSH levels in the PCOS group this reduction of FSH was not found to be significant when compared to the EuHyperA group. The significantly increases levels of 17-OHP, Δ4-ASD and TT throughout the test in the PCOS group correlate with a deregulation in LH and FSH levels, which is frequently observed within the literature [25], and may lead an enhanced response to GnRH agonist.

In brief, as confirmed by inspection of the percent changes of hormone levels after each of the two Dex regimens, the higher post-Dex levels of 17-OHP, Δ4-ASD and TT are associated with a lower degree of suppression. In other terms, EuHyperA women have a greater sensitivity to the suppressive action of Dex on adrenal steroidogenesis. Within either group, absence of FOH is associated with a greater sensitivity to adrenal suppression. When FOH is present, women with HR-PCOS reveal a lower degree of suppression of steroidogenesis as evidenced by 17-OHP levels, which translate into a significant difference in androgen levels. This evidence highlights an interesting aspect, described as a possible higher production of androgen species from the ovary of PCOS patients in response to Dex.

Finally, a comparison of our study with the two pertinent studies of the literature related to EuHyperA [8] or PCOS [3] is described in the **Supplementary Material** with the assistance of **Table S-1 and Table S-2**.

Our study has some limitations. First, it is retrospective in nature. Secondly, technical advancements in ultrasonography may lead to inaccuracies in the ovarian evaluation that had been performed 3 decades earlier. In the present study, the patients with PCOS were not stratified according to their phenotype. Thirdly, the differences in the number of patients between the EuHyperA and the PCOS groups may reduce the significant differences observed. A similar consideration also applies to the comparison between the HR and between the NR subgroups.

Moreover, it would be interesting to ascertain different PCOS phenotypes feature different levels of adrenal suppression by Dex, and responses of hormones to the GnRH stimulation test.

Conclusions

In the present study we retrospectively compared women with EuHyperA and PCOS for three important parameters: adrenal suppression by two regimens of Dex, hormone responses to GnRH-agonist (buserelin), and OGTT. The results of this study demonstrate that women with PCOS have an increased rate of FOH in addition to significantly higher levels of 17-OHP, Δ4-ASD, and TT after Dex suppression of adrenal glands. It appears that women with PCOS have a lower degree of adrenal suppression and a likely increased ovarian androgen production. This evidence is supported by an increased ovarian volume of patients with PCOS, particularly in HR subgroup, thus indicating an overactivity in androgen production in the theca cells. In contrast, EuHyperA patients were more sensitive to the Dex administration. with a higher degree of suppression of androgen production.

Moreover, AUC 0–120 min insulinemia, in addition to insulin resistance, were slightly increased in PCOS compared to EuHyperA. The differences in metabolic patterns were also observed in the comparison between HR and NR subgroups in both PCOS and EuHyperA groups, specifically concerning insulin levels and the presence of insulin-resistance phenomena. This may correlate with the different response observed in the study groups, as insulin deregulation frequently leads to increased androgen production in the ovary of patients with PCOS. To conclude, the overall evaluation of the parameters analyzed in the study indicate similar values between the two study groups, may permit the hypothesis that EuHyperA could represent a mild form of PCOS, lacking metabolic alteration and menstrual cycle disorders.

Funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sector.

CRediT authorship contribution statement

Salvatore Benvenga: Writing – original draft, Writing – review & editing. **Michele Russo:** Writing – review & editing. **Gianpiero Forte:** Writing – review & editing. **Vittorio Unfer:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Vittorio Unfer reports a relationship with Lo.Li. pharma that includes: employment. M.R., G.F and V.U. are employees of Lo.Li Pharma s.r.l. All other authors have no conflicts of interest to declare. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.].

Data availability

Data will be made available on request.

Aknowledgements

We acknowledge Dr. Samuel H Myers for editing and revising the English language of the present manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcte.2024.100368.

REFERENCES

[1] Ehrmann DA, Rosenfield RL, Barnes RB, Brigell DF, Sheikh Z. Detection of functional ovarian hyperandrogenism in women with androgen excess. N Engl J Med 1992;327(3):157–62. https://doi.org/10.1056/nejm199207163270304.
[2] Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004; 19(1): 41-7, 10.1093/humrep/deh098.
[3] Pasquali R, Patton L, Pocognoli P, Cognigni GE, Gambineri A. 17-hydroxyprogesterone responses to gonadotropin-releasing hormone disclose distinct phenotypes of functional ovarian hyperandrogenism and polycystic ovary syndrome. J Clin Endocrinol Metab 2007;92(11):4208–17. https://doi.org/10.1210/jc.2007-0870.
[4] Pappalardo MA, Russo GT, Pedone A, Pizzo A, Borrielli I, Stabile G, et al. Very high frequency of the polymorphism for the insulin receptor substrate 1 (IRS-1) at codon 972 (glycine972arginine) in Southern Italian women with polycystic ovary syndrome. Horm Metab Res 2010;42(8):575–84. https://doi.org/10.1055/s-0030-1249020.

[5] Ambrosi B, Re T, Passini E, Bochicchio D, Ferrario R. Value of buserelin testing in the evaluation of hirsute women. J Endocrinol Invest 1996;19(4):204–9. https://doi.org/10.1007/bf03349868.
[6] Ibañez L, Hall JE, Potau N, Carrascosa A, Prat N, Taylor AE. Ovarian 17-hydroxyprogesterone hyperresponsiveness to gonadotropin-releasing hormone (GnRH) agonist challenge in women with polycystic ovary syndrome is not mediated by luteinizing hormone hypersecretion: evidence from GnRH agonist and human chorionic gonadotropin stimulation testing. J Clin Endocrinol Metab 1996;81(11):4103–7. https://doi.org/10.1210/jcem.81.11.8923867.
[7] Chang PL, Lindheim SR, Lowre C, Ferin M, Gonzalez F, Berglund L, et al. Normal ovulatory women with polycystic ovaries have hyperandrogenic pituitary-ovarian responses to gonadotropin-releasing hormone-agonist testing. J Clin Endocrinol Metab 2000;85(3):995–1000. https://doi.org/10.1210/jcem.85.3.6452.
[8] Carmina E, Lobo RA. Do hyperandrogenic women with normal menses have polycystic ovary syndrome? Fertil Steril 1999;71(2):319–22. https://doi.org/10.1016/s0015-0282(98)00455-5.
[9] Rosen MP,Cedars MI. *Greenspan's Basic & Clinical Endocrinology*; DS David G. Gardner, Ed.; McGraw-Hill Medical: New York, 2007; Issue: Female Reprodutive Endocrinology, pp. 502-61.
[10] Teede HJ, Tay CT, Laven JJE, Dokras A, Moran LJ, Piltonen TT, et al. Recommendations From the 2023 International Evidence-based Guideline for the Assessment and Management of Polycystic Ovary Syndrome. J Clin Endocrinol Metab 2023;108(10):2447–69. https://doi.org/10.1210/clinem/dgad463.
[11] Unfer V, Dinicola S,Russo M. A PCOS Paradox: Does Inositol Therapy Find a Rationale in All the Different Phenotypes? Int J Mol Sci 2023; 24(7), 10.3390/ijms24076213.
[12] Lebbe M, Woodruff TK. Involvement of androgens in ovarian health and disease. Mol Hum Reprod 2013;19(12):828–37. https://doi.org/10.1093/molehr/gat065.
[13] Hajam YA, Rather HA, Neelam, Kumar R, Basheer M,Reshi MS. A review on critical appraisal and pathogenesis of polycystic ovarian syndrome. Endocrine and Metabolic Science 2024; 14100162, https://doi.org/10.1016/j.endmts.2024.100162.
[14] Wang K, Li Y,Chen Y. Androgen excess: a hallmark of polycystic ovary syndrome. Front Endocrinol (Lausanne) 2023; 141273542, 10.3389/fendo.2023.1273542.
[15] Fulghesu AM, Angioni S, Frau E, Belosi C, Apa R, Mioni R, et al. Ultrasound in polycystic ovary syndrome–the measuring of ovarian stroma and relationship with circulating androgens: results of a multicentric study. Hum Reprod 2007;22(9):2501–8. https://doi.org/10.1093/humrep/dem202.
[16] Mansour A, Hashemi Taheri AP, Moradi B, Mohajeri-Tehrani MR, Qorbani M, Ghorbani Pashakolaee S, et al. Ovarian volume, not follicle count, is independently associated with androgens in patients with polycystic ovary syndrome. BMC Endocr Disord 2022;22(1):298. https://doi.org/10.1186/s12902-022-01224-y.
[17] Lim SS, Davies MJ, Norman RJ, Moran LJ. Overweight, obesity and central obesity in women with polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update 2012;18(6):618–37. https://doi.org/10.1093/humupd/dms030.
[18] Daan NM, Louwers YV, Koster MP, Eijkemans MJ, de Rijke YB, Lentjes EW, et al. Cardiovascular and metabolic profiles amongst different polycystic ovary syndrome phenotypes: who is really at risk? Fertil Steril 2014;102(5):1444–51e3.
[19] Livadas S, Anagnostis P, Bosdou JK, Bantouna D, Paparodis R. Polycystic ovary syndrome and type 2 diabetes mellitus: A state-of-the-art review. World J Diabetes 2022;13(1):5–26. https://doi.org/10.4239/wjd.v13.i1.5.
[20] Tosi F, Villani M, Migazzi M, Faccin G, Garofalo S, Fiers T, et al. Insulin-mediated substrate use in women with different phenotypes of PCOS: the role of androgens. J Clin Endocrinol Metab 2021;106(9):e3414–25. https://doi.org/10.1210/clinem/dgab380.
[21] de Zegher F, Ibáñez L. Leader vs follower in the tango of polycystic ovary syndrome: Insulin resistance vs androgen excess. Acta Obstet Gynecol Scand 2024. https://doi.org/10.1111/aogs.14802.
[22] Unfer V, Kandaraki E, Pkhaladze L, Roseff S, Vazquez-Levin MH, Laganà AS, et al. When one size does not fit all: Reconsidering PCOS etiology, diagnosis, clinical subgroups, and subgroup-specific treatments. Endocrine and Metabolic Science 2024; 14100159, https://doi.org/10.1016/j.endmts.2024.100159.
[23] Chen W,Pang Y. Metabolic Syndrome and PCOS: Pathogenesis and the Role of Metabolites. Metabolites 2021; 11(12), 10.3390/metabo11120869.
[24] Qin KN, Rosenfield RL. Role of cytochrome P450c17 in polycystic ovary syndrome. Mol Cell Endocrinol 1998;145(1–2):111–21. https://doi.org/10.1016/s0303-7207(98)00177-4.
[25] Ting Z, Xinghua T, Xiao X, Lingchuan L, Xiaomei W, Tao Y. The impact of androgen levels on serum metabolic profiles in patients with polycystic ovary syndrome. Gynecol Endocrinol 2024;40(1):2352136. https://doi.org/10.1080/09513590.2024.2352136.