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Androstenedione response to recombinant human FSH is the most valid predictor of the number of selected follicles in polycystic ovarian syndrome: (a case-control study)

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Abstract

Background: We aimed to test the hypothesis that the correlation of the changes in the blood Androstenedione (A_4) levels to the number of selected follicles during ovulation induction with low-dose recombinant human follicle stimulating hormone (rhFSH) is as strong as the correlation to changes in the blood Estradiol (E_2) levels in polycystic ovary syndrome (PCOS).

Methods: Prospective Case-control study conducted from October 2014 to January 2016. 61 non-PCOS control (Group I) and 46 PCOS (Group II) patients treated with the chronic low-dose step up protocosl with rhFSH. A_4, E_2, progesterone blood levels and follicular growth were monitored.. Univariate and hierarchical multivariable analysis were performed for age, BMI, HOMA-IR, A_4 and E_2 (with the number of selected follicles as the dependent variable in both groups). ROC analysis was performed to define threshold values for the significant determinants of the number of selected follicles to predict cyle cancellations due to excessive ovarian response.

Results: The control group (Group I) was comprised of 61 cycles from a group of primary infertile non-PCOS patients, and the study group (Group II) of 46 cycles of PCOS patients. The analysis revealed that the strongest independent predictor of the total number of selected follicles in Group I was the E_2(AUC) (B = 0.0006[0.0003-0.001]; $P < 0.001$); whereas for Group II, it was the A_4 (AUC) (B = 0.114[0.04-0.25]; $P = 0.01$). Optimum thresholds for the A_4 related parameters were defined to predict excessive response within Group II were 88.7%, 3.1 ng/mL and 5.4 ng*days for the percentage increase in A_4, the maximum A_4 value and area under the curve values for A_4, respectively.

Conclusion: A_4 response to low-dose rhFSH in PCOS has a stronger association with the number of follicles selected than the E_2 reponse. A_4 response preceding the E_2 response is essential for progressive follicle development. Monitoring A_4 rather than E_2 may be more preemptive to define the initial ovarian response and accurate titration of the rhFSH doses.

Trial registration: The study was registered as a prospective case-control study in the ClinicalTrials.gov registry with the identifier NCT02329483.

Keywords: Androgens, Androstenedione, Polycystic ovary syndrome, Ovulation induction, Folliculogenesis, Human FSH, Gonadotropins

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Background

Oligoovulation related to polycystic ovarian syndrome is treated with ovulation induction medications [1]. These patients have an increased risk of excessive ovarian response which is closely associated with the number of selected follicles [2]. Therefore, milder protocols have been developed [3]. In PCOS cases, the estradiol response to gonadotropin treatment is delayed and discordant with the visualized follicular responses [3]. Androstenedione (A_4), mostly synthesized in the ovaries is a precursor of E_2 [4, 5].

In this study, we aimed to test the hypothesis that the cumulative changes in A_4 during ovulation induction with low dose rhFSH in PCOS cases are correlated to the number of selected follicles (follicles sized ≥12 mm) comparable to the the cumulative changes in E_2.

Methods

This is a prospective case-control study conducted between October 2014 and January 2016. Ethical permission was obtained from the Bagcilar Research and Training Hospital Research Ethics Committee. It was recorded as a prospective case-control study in the ClinicalTrials.gov registry with the identifier NCT02329483. The study was conducted in accordance with the Declaration of Helsinki. Informed consent for participation was obtained from all patients.

Study setting

The study was conducted at the Bagcilar Research and Training Hospital Gynecology and Obstetrics Department, Infertility Section. Cycle monitoring was done with folliculometry with transvaginal sonography, E_2, P_4 and A_4 measurements.

Study population

A total of 107 cycles of 61 Control-nonPCOS infertile (Group I) and 46 Study-PCOS infertile (Group II) women were included in the study. The study group was comprised of patients with anti-Müllerian hormone (AMH) levels ≥5 ng/mL (which is equivalent to PCOM (polycystic ovary morphology sonographically confirmed) [6–8]. PCOS was defined as the copresence of at least one of two of the following criteria combined with the PCOM; *(1): oligoamenorrhea-OA*: cycle length > 35 days, *(2): hyperandrogenism (HA)*: presence of clinical findings including hirsutism defined as the presence of coarse [long/pigmented] terminal hair over the most commonly encountered three or more regions within the Modified Ferriman-Gallwey Score System (the buttocks/perineum, sideburn, and neck areas which contributed greatly to the score in the geographic locale where this study was conducted) with or without elevated blood testosterone levels (>0.5 ng/mL) [7–12]. The control group was comprised of unexplained primary/secondary infertile women with AMH levels <5 ng/mL, not displaying any clinical findings associated with hyperandrogenism (HA) and with regular menstrual cycles. The inclusion criteria for both groups included ages within 20-35, with normal spermiograms or with mild male factor infertility (i.e.: male partners with only one the following abnormalities: sperm counts being lower than 20 million/ml *or* showing a normal morphology quotient of less than 4% *or* having a sperm motility lower than 40%; *AND* with post-wash total motile sperm counts equal to or higher than 5 million/ml), normal anatomic findings with the hysterosalpingography (no bilateral tubal obstruction or Müllerian anomalies), hormonally eugonadotropic, normal blood prolactin/thyroid-stimulating hormone (TSH) levels and being planned for controlled ovarian hyperstimulation and intrauterine insemination treatment. Exclusion criteria included: diabetes mellitus, BMI < 20 or >30, hypo or hypergonadotropism, other causes for hyperandrogenism, ≥2 abortions or ectopic pregnancy, additional medical disorders, ovarian cysts or previous pelvic surgery.

Controlled ovarian hyperstimulation and intrauterine insemination

Ovulation induction was conducted with follitropin alpha (Gonal-f Multidose 450 IU; Merck-Serono-Turkey) starting with a dose of 37.5 U/day or 50 U/day as described by Homburg et al. [2]. Ovulation induction was started on the 3rd or the 4th day of a menstrual cycle having early cycle blood E_2 levels <50 pg/mL and blood Progesterone levels <0.5 ng/mL and in the absence of any ovarian residual follicles larger than 15 mm to rule out the presence of a corpus luteum or any other cystic ovarian structure which could require further clarification. If a primary follicle response characterised by the appearance of a selected growing follicle of ≥10 mm and a rise ≥25% in blood E_2 levels was not observed despite 14 days of rhFSH stimulation, the initial dose was increased initially to 75 U/day and +37.5 U/day, weekly at each additional incremental step (i.e. 112.5, 150 U/day). Blood E_2, P_4, and A_4 levels were measured and follicle growth was monitored with transvaginal sonography at every visit (every 2-3 days) starting on day 2 or 3 of the cycles. Once 1 or 2 mature follicles ≥18 mm were observed, rhCG (Ovitrelle 150 μg; Merck-Serono-Turkey) s.c. was administered. Sperm washing and intrauterine insemination were carried within [36th–40th] hours. On the 15th day postinsemination, blood beta-hCG levels were measured and conception confirmed if the beta-hCG blood level was higher than 20mIU/mL.

Cycle cancellation policy

Cycle cancellations were due to excessive ovarian responses (more than 2 selected follicles ≥16 mm or blood E_2 level > 1500 pg/mL on the rhCG trigger day), no

ovarian response despite dose step-up and stimulation for 28-30 days, or premature luteinisation (P_4 blood level ≥ 1.3 ng/mL).

Laboratory analysis of blood samples

Blood samples for hormone measurement were collected from the antecubital vein with a single puncture at every visit during ovulation induction. Samples were collected in a sterile tube and transferred to the lab on the same day. All except the A_4 blood level measurement results were reported to the physicians on the afternoon of the same day. A_4 blood levels were available 7-10 days later, and did not provide any guidance to management. A colorimetric ELISA assay (Abcam-USA; Kimera Istanbul-Turkey) was used to measure A_4 levels. Measurements of AMH were made by using the AMH/MIS enzyme-linked immunosorbent assay. Testosterone values were assayed with the competitive immunoenzymatic colorimetric method. The serum FSH, luteinising hormone (LH), TSH, E_2, and prolactin levels were measured using a chemiluminescent microparticle immunoassay.

Data analysis

Univariate parametric tests were used for group comparisons. Significance was defined as a *P*-value <0.05. A_4 (AUC) was calculated as the sum of the areas of trapezoids. Primary A_4 response during ovulation induction was considered when a rise of ≥25% was observed in the basal A_4 level. HOMA-IR (Homeostatic Model Assessment of Insulin Resistance) Index was calculated for each patient by using an online calculator.

Hierarchial multivariable regression analysis was conducted in both groups to study in a three level linear regression model the contribution of *three sets of independent variables* including the (Model 1) age, BMI and HOMA-IR variables; (Model 2) the E_2(AUC) values and (Model 3) (the androstenedione related variables: the primary blood androstenedione level and the A_4 (AUC) values); stepwise, defining *changes in R^2values (ΔR^2)* representing the additional effect of each of these newly added independent variable sets, on *the total number of selected follicles*. The SPSS 20.0 and Microsoft Excel 2010 were used.

ROC analysis was performed to define threshold values of the significant determinants of the number of selected follicles to predict cyle cancellations due to excessive ovarian response.

Results

A total of 107 cycles of infertile women Group I: 61 non-PCOS and Group II: 46 PCOS were followed in 16-months. The PCOS phenotypes were: PCOM (polycystic ovarian morphology:AMH ≥ 5 ng/mL)/OA (oligo/amenorrhea): 4 patients; PCOM/HA (Hyperandrogenism): 27 patients; PCOM/HA/OA: 15 patients. Mild male factor infertility was present in (22/61) 36% of Group I and (15/46) 32.6% in Group II (*P* = 0.5). The HOMA-IR score was ≥4.5 in 6/61 (9,8%) and 9/46 (19,6%) of patients in Groups I and II, respectively. The patient characteristics are summarised in (Table 1). There were 6/61 (9.8%) and 9/46 (19.5%) conceptions in Groups I and II, respectively. The comparison of hormonal characteristics among the conceived and the nonconceived subjects within Groups I and II are summarized in (Table 2). No conception was achieved in the absence of a primary androstenedione response earlier than the primary estradiol response. Cancelled cycles were not included in this comparison.

Table 1 Cycles analysed in this study

	Group I (Control Group)	Group II (PCOS Group)
Age	29.8 ± 0.6	28.7 ± 0.6
Duration of infertility (years)	3.5 ± 0.3	3.4 ± 0.4
BMI (kg/m^2)[a]	25 ± 0.7	27.7 ± 0.6
AMH(ng/mL)[a]	2.4 ± 0.2	9 ± 0.9
HOMA[a]	2.8 ± 0.4	3.8 ± 0.4
FSH (mIU/ml)[a]	7.2 ± 0.3	6.3 ± 0.2
LH (mIU/ml)[a]	6.5 ± 0.5	9.8 ± 0.6
TSH (μIU/mL)	2.7 ± 0.2	3.3 ± 0.7
PRL (ng/ml)	20.9 ± 1.2	22 ± 1.9
Initial Dose (IU/day)	70 ± 4.1	65 ± 3.7
Cycle Length (days)	12.2 ± 4.1	17 ± 0.6
Primary follicular/E_2 response day[a]	6.7 ± 0.4	9.4 ± 0.4
EM at trigger day (mm)	9.9 ± 0.3	9.4 ± 0.3
Maximum E_2 (pg/dl)	453 ± 42.1	556.9 ± 89.3
Follicles >16 mm (n)	1.1 ± 0.1	1.4 ± 0.2
Follicles 12-16 mm (n)	1.2 ± 0.2	1.6 ± 0.3
Total number of follicles	1.9 ± 0.2	2.2 ± 0.3
P_4 at trigger day (ng/mL)	0.7 ± 0.1	0.8 ± 0.1
Day 3 Total Testosterone (ng/mL)[a]	0.34 ± 0.28	0.87 ± 0.3
Primary androstenedione level (ng/mL)[a]	0.9 ± 0.1	1.4 ± 0.1
Primary androstenedione respond day[a]	6.2 ± 0.3	7.4 ± 0.4
Time Lag ($A_4 \rightarrow E_2$) initial responses (days)[a]	0.5 ± 0.4	2 ± 0.5
Maximum A_4(ng/mL)[a]	1.8 ± 0.1	2.6 ± 0.3
Rise in A_4 (%)[a]	66.5 ± 6.9	84.1 ± 7.4
A_4 (AUC) (ng*days)[a]	3.6 ± 0.3	5.5 ± 1.3
A_4 on the trigger day (ng/mL)[a]	1.3 ± 0.1	2.5 ± 0.4

PCOS polycystic ovarian syndrome, *AMH* Anti-Müllerian hormone, *HOMA* homeostasis model for assesment of insulin resistance, *FSH* follicle-stimulating hormone, *LH* luteinising hormone, *TSH* thyroid-stimulating hormone, E_2 estradiol, *PRL* prolactin, *EM* endometrial thickness, P_4 progesterone, *AUC* area under the curve, A_4 androstenedione

[a]$P < 0.05$

Table 2 Comparison of the hormonal characteristics among the conceived and nonconceived subjects

	Group I (Control Group)		Group II (PCOS Group)	
	Did not conceive	Conceived	Did not conceive	Conceived
Primary A_4[a]	0,88 ± 0.45	0,96 ± 0.2	1,39 ± 0.08	1.4 ± 0.33
A_4 (AUC)[b]	3,06 ± 2.8	2.8 ± 1.3	4,2 ± 0.7	10.7 ± 7.1
E_2(AUC)[c]	1916,6 ± 206.3	2942.7 ± 1058.4	2654,2 ± 677.3	4212.7 ± 767.2
AMH[d]	2,53 ± 1.2	3.0 ± 0.41	9.23 ± 1	8.6 ± 1.5
HOMA-IR[e]	3,08 ± 0.3	2.4 ± 0.6	3,2 ± 0.34	2.22 ± 0.33
Time Lag A_4-E_2Response	−0,92 ± 0.46	−0,67 ± 0.4	−0,23 ± 0.11[af]	−3.79 ± 0.86[af]

[a]The initial blood androstenedione level (ng/mL)
[b]Area under the curve value for Androstenedione (ng*days)
[c]Area under the curve value for estradiol (pg*days)
[d]Antimüllerian hormone (ng/mL)
[e]Homeostasis Model for Assessment of Insulin Resistance
[f]$P<0.05$

Nineteen cycles were cancelled due to excessive response (n = 8), no response (n = 6), or premature luteinisation (n = 5). In those cycles cancelled due to no response, there was no primary A_4 response.

Univariate analysis revealed that the primary, maximum, and AUC values for A_4 and the primary blood testosterone levels were all higher in Group II than those in Group I (Table 1).

Plateauing or decreasing A_4 levels before the trigger day were observed in 33/61 (54,1%) of completed cycles in Group I and 14/46 (30,4%) of cycles in Group II. None of these made any significant difference in the basic characteristics or outcome parameters.

The correlation of A_4 parameters with the selected follicle numbers and E_2 (AUC) values are summarised in Figs. (1 and 2; and Tables (3 and 4): The A_4(AUC) was

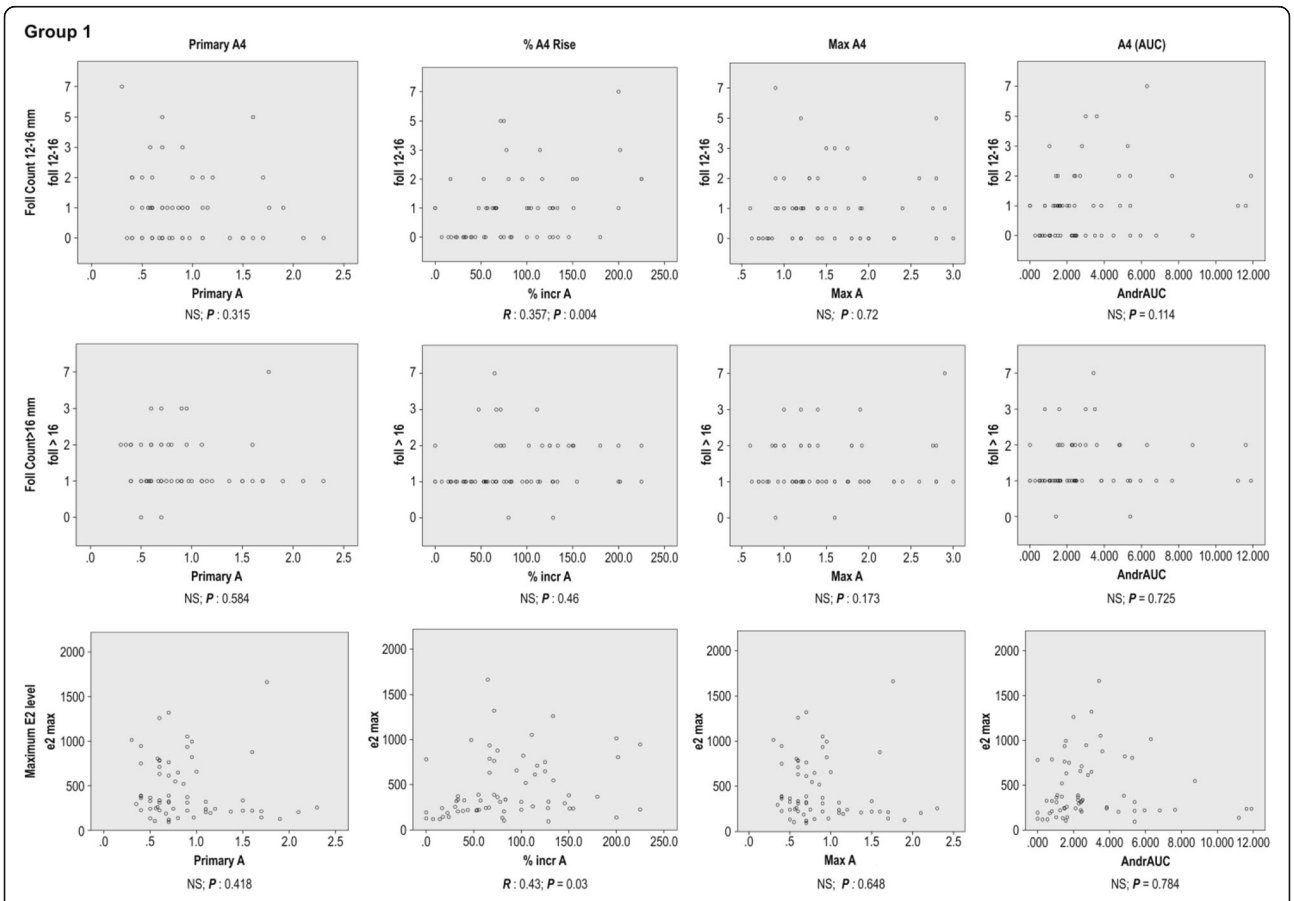

Fig. 1 Correlation of A_4 related parameters with cycle outcome in Group I

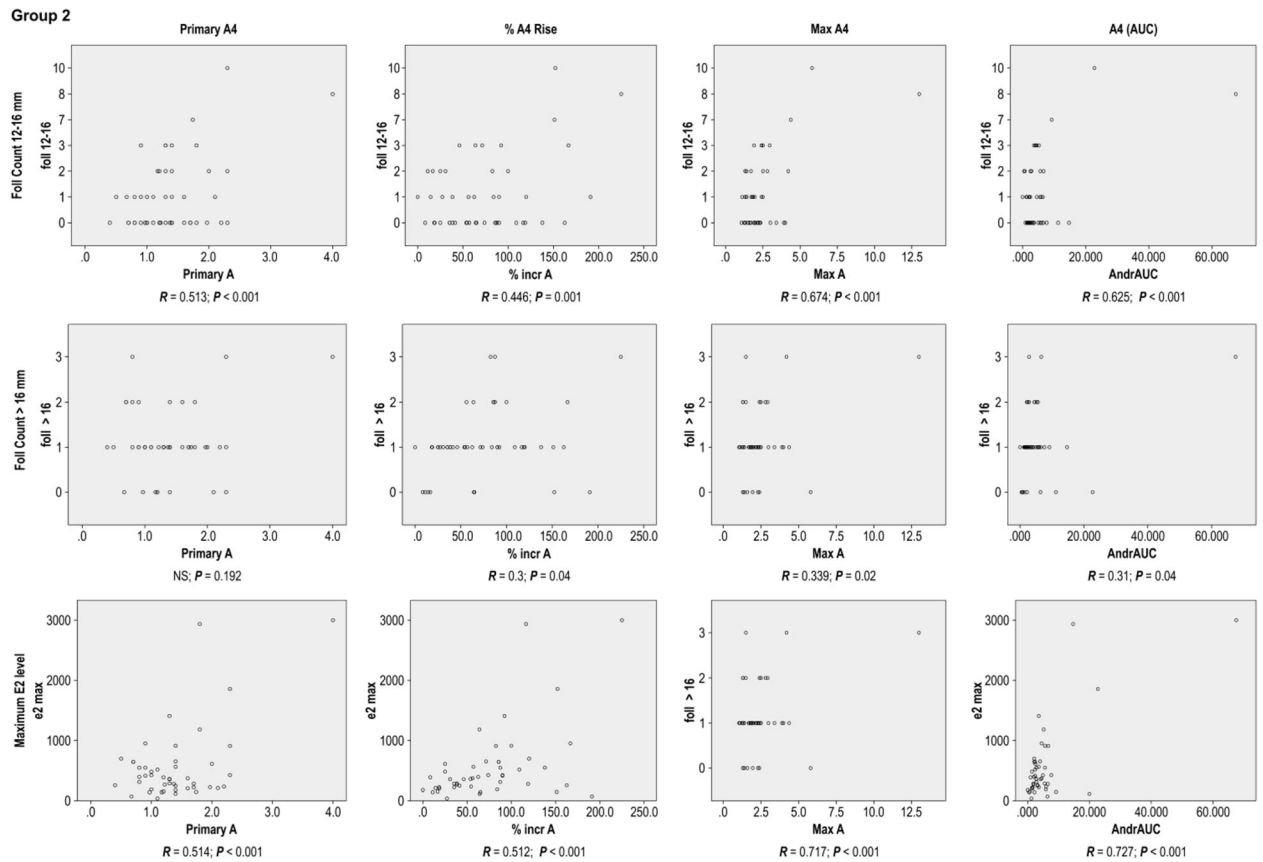

Fig. 2 Correlation of A_4 related parameters with cycle outcome in Group II

correlated with the number of selected follicles and E_2(AUC) in Group II, but not correlated with any one of these parameters in Group I The primary A_4 levels were correlated to the primary testosterone levels in both Groups I and II.

Hierarchical multivariable regression analysis was conducted seperately for *Groups I and II*. The findings of the analysis are summarized in Tables 5(a,b) and 6(a,b). The total number of selected follicles (≥12 mm) was the dependent (outcome) variable. The effect of age, BMI and HOMA-IR on the total number of selected follicles were not significant in either Group I or II. In Group I: the estradiol (AUC) was *the strongest independent factor* (B = 0.0006[0.0003-0.001]; $P < 0.001$), *whereas* in Group II: the A_4(AUC) (B = 0.114 [0.04-0.25]; $P = 0.01$) was *the strongest independent factor effecting the total number of selected follicles.*

Optimum thresholds for the A_4 related parameters were defined to predict excessive response within Group II

Table 3 Correlation of A_4 parameters with cycle outcome parameters in Group I (Control Group)

R^a	Primary A_4	increase A_4%[b]	Max A_4	A_4 AUC
Foll 12-16[d]	NS	0.36[Y]	NS	NS
Foll >16[c]	NS	NS	NS	NS
Maximum E_2	NS	0.43[Y]	NS	NS
Total Foll[e]Selected	*NS*	*0.32[Y]*	*NS*	*NS*

E_2 = Estradiol; A_4 = Androstenedione; AUC = Area under the curve;
Foll = Number of follicles
[Y]$P < 0.005$
[a]Pearson Correlation constant
[b]Percentage rise in the A_4 level
[c]number of follicles >16 mm
[d]number of follicles 12-16 mm
[e]number of follicles >12 mm

Table 4 Correlation of A_4 parameters to the cycle outcome parameters in Group II (PCOS Group)

R^a	Primary A_4	increase A_4%[b]	Max A_4	A_4 AUC
Foll 12-16[d]	0.513[Y]	0.446[Y]	0.674[Y]	0.625[Y]
Foll >16[c]	NS	0.3	0.339[Y]	0.31[Y]
Maximum E_2	0.514[Y]	0.512[Y]	0.717[Y]	0.727[Y]
Total Foll Selected[e]	*0.542[Y]*	*0.5[Y]*	*0.73[Y]*	*0.673[Y]*

E_2 = Estradiol, A_4 = Androstenedione, AUC = Area under the curve,
Foll = Number of follicles
[a]Pearson Correlation constant
[b]Percentage rise in the A_4 level
[c]number of follicles >16 mm
[d]number of follicles 12-16 mm
[e]number of follicles >12 mm
[Y]A significant correlation; $P<0.05$

Table 5 Hierarchical multivariable regression analysis of independent variables in Group I (Control Group)

Model Summary for Group I				
(A): Model Summary				
Models		Change Statistics		
	R Square	R Square Change	F Change	Sig. F Change
1[a]	0,07	0,07	1,1	NS
2[b]	***0,42***	***0,35***	***25,4***	***<0.001***
3[c]	0,44	0,01	0,5	NS
(B): Coefficients				
Models		Coefficients		
		B	Std. Error	P
1[a]	(Constant)	3,49	1,78	0,06
	Age	0,0004	0,05	0,99
	BMI	−0,02	0,04	0,59
	HOMA-IR	−0,19	0,13	0,15
2[b]	(Constant)	−0,46	1,63	0,78
	Age	0,06	0,04	0,17
	BMI	−0,01	0,03	0,75
	HOMA-IR	0,03	0,11	0,76
	E_2(AUC)	***0,0006***	***0,0001***	***<0.001***
3[c]	(Constant)	−0,50	1,65	0,77
	Age	0,06	0,04	0,20
	BMI	−0,02	0,03	0,66
	HOMA-IR	−0,03	0,13	0,83
	E_2 (AUC)	***0,0006***	***0,0001***	***<0.001***
	Primary A	0,48	0,49	0.68
	A_4(AUC)	0,01	0,07	0,85

[a]Model 1: including the independent variables Age, BMI, HOMA-IR
[b]Model 2: adding the independent variable E2 (AUC) to the previous Model 1
[c]Model: adding the independent variables Primary A and A4(AUC) to the previous Model 2

Table 6 Hierarchical multivariable regression analysis of independent variables in Group II (PCOS Group)

Model Summary for Group 2				
(A): Model Summary				
Models		Change Statistics		
	R Square	R Square Change	F Change	Sig. F Change
1[a]	0,05	0,05	0,3	0.8
2[b]	***0,49***	***0,44***	***16,6***	***<0.001***
3[c]	***0,63***	***0,14***	***15,1***	***0.001***
(B): Coefficients				
Models		Coefficients		
		B	Std. Error	P
1[a]	(Constant)	5,68	3,37	0,10
	Age	−0,05	0,08	0,57
	BMI	−0,07	0,10	0,50
	HOMA-IR	−0,10	0,21	0,64
2[b]	(Constant)	6,14	2,52	0,02
	Age	−0,06	0,06	0,36
	BMI	−0,12	0,08	0,13
	HOMA-IR	−0,08	0,15	0,62
	E_2(AUC)	***0,002***	***0,001***	***<0,01***
3[c]	(Constant)	4,62	2,43	0,07
	Age	−0,01	0,06	0,89
	BMI	−0,14	0,07	0,05
	HOMA-IR	−0,06	0,14	0,67
	E_2 (AUC)	0,0005	0,0003	0,34
	Primary A	0,57	0,60	0,35
	A_4(AUC)	***0,114***	***0,03***	***0.01***

[a]Model 1: including the independent variables Age, BMI, HOMA-IR
[b]Model 2: adding the independent variable E2 (AUC) to the previous Model 1
[c]Model: adding the independent variables Primary A and A4(AUC) to the previous Model 2

combined were as in Table 7: 88.7%, 3.1 ng/mL and 5.4 ng*days for the percentage increase in A_4, the maximum A_4 value and area under the curve values for A_4, respectively.

Discussion

In this study, we observed that in PCOS, the cumulative A_4 response to low-dose rhFSH is a more valid measure of the number of selected follicles than the cumulative Estradiol (E_2) response [13]. The early and midfollicular A_4 variations are more critical determinants than the late follicular variations (following follicle selection), because drops or plateauing observed in A_4 in the late stages did not influence cycle outcome. The A_4 respond to rhFSH was earlier than that of E_2 in cycles with progressive follicular growth and conception.

In six cycles cancelled due to lack of response to rhFSH, there was no A_4 response. In contrast, in four of the eight cycles cancelled due to excessive response, a dosage step-up had been made due to lack of E_2 response, while there had already been an initial A_4 response. If this corrective information could have been taken into account, an unnecessary step-up could have been avoided.

PCOS is the most common cause of anovulatory infertility, and is reported to comprise 15.3% of the women living in the geographic region where this study was conducted [14]. PCOS is characterised by three main elements: follicular growth arrest, hyperandrogenism and excessive folliculogenesis [15].

The chronic low-dose step up protocol used for ovulation induction in PCOS patients requires up to 14 days of rhFSH treatment to overcome a temporary ovarian refractoriness ending with follicle selection [16]. The follicular growth response initiated by rhFSH in the granulosa cell component when treated with

Table 7 ROC analysis of Androstenedione related parameters (maximum A4, percentage increase in A4 and A4 area under the curve) to define optimum threshold parameters to predict cycle cancellations in Group II

	Area Under Curve ± SEM [5-95p]	Optimum Threshold	Sensitivity (%)	Specificity (%)
A_4 percentage increase	0.73 ± 0.07 [0.6-0.87]	88.7%	75	72
Maximum A_4	0.78 ± 0.19 [0.66-0.9]	3.1 ng/mL	75	74
A_4 (AUC)	0.78 ± 0.06 [0.67-0.9]	5.4 ng*day	70	72

rhFSH is propagated to the theca cell component. During this initial response, in PCOS patients, the reversal of the FSH/LH effect in favour of FSH and aromatisation may not be as hormonally evident with rising E_2 blood levels as the thecal androgen response, especially at earlier stages. Granulosa cells of the antral follicle at this stage normally respond to this early rise in A_4 by increasing their aromatase activities, which is strongly counteracted by high AMH levels in the follicular microenvironment of the PCOS follicles, analogous to the AMH-FSH counteraction at later stages of follicular growth [17]. Thus, the transient follicular growth arrest is observed at this early stage due to the AMH-androgen counteraction [18, 19]. High androgen and AMH concentrations also contribute to the microenvironment that fosters excessive folliculogenesis and follicular growth arrest [11, 20–23].

Basal androgen blood levels measured at the beginning or during induction cycles have been reported in various studies in low responders, patients with diminished ovarian reserves, or normal responders, but not in PCOS cases. Ferrario et al. showed in a group of older women with low response that A_4 levels measured at the beginning of IVF cycles were predictors of positive outcome [24]. Similarly, Sun et al. have shown in a study of 1413 infertile women going through their first cycle of IVF that the testosterone blood levels measured at the beginning of the treatment cycles were predictors of the number of follicles larger than 14 mm on the day of hCG trigger, but were not predictors of conception [25]. It would be interesting and supplementary to monitor the testosterone response to rhFSH during ovulation induction cycles in prospective observational studies.

Our study had some *potential causes of bias and limitations* which need to be addressed. From the perspective of the Rotterdam 2003 definition of PCOS, cases with the nonPCOM/HA/OA (defined as phenotype B) may be being underrepresented in the infertile PCOS patients group (Group II) [26]. Another limitation was that the BMI's in *Group II* were slightly, but significantly higher than *Group I*. However, in the hierarchical multivariable regression conducted, BMI as well as age and HOMA-IR were included in a separate model and their effects on the total number of selected follicles were found to be insignificant in both *Groups I and II*.

Conclusion

The findings in this clinical study suggest that the reactive rise in androstenedione in the early follicular phase is a better predictor of the number of follicles selected than the conventionally used reactive rise in estradiol in PCOS cases. The longer/higher is the increase in its blood levels, the more are the follicles joining the growing cohort with an increasing risk of excessive ovarian response. On the other hand, androstenedione is an earlier and more reliable marker of the initial ovarian response to gonadotropins and this earlier response may be essential for progressive follicle growth and possibly a conception in an ovulation induction and intrauterine insemination cycle (using rhFSH) in PCOS. It still needs to be further studied in prospective studies encompassing induction cycles managed mainly with A_4 monitoring, to provide stronger evidence if androstenedione monitoring provides a more valid and useful information to indicate ovarian response and if an earlier androstenedione response is associated with conception.

Abbreviations

A4: Androstenedione; AMH: Antimüllerian hormone; AUC: Area under the curve; beta-hCG: beta human chorionic gonadotroopin; BMI: Body mass index; E2: Estradiol.; FSH: Follicle stimulating hormone; HA: Hyperandrogenism; HOMA-IR: Homeostatic model assessment of insulin resistance; IVF: In vitro fertilization; OA: Oligo/amenorrhea; P4: Progesterone; PCOM: polycystic ovarian morphology; PCOS: Polycystic ovary syndrome; rhFSH: Recombinant human follicle stimulating hormone; ROC: Receiver operating curve; TSH: Thryoid stimulating hormone

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Availability of data and materials

The dataset supporting the conclusion of this article is included within the article.

Authors' contributions

SEO conceptualized and suggested a plan for the study. TY then joined the study group and helped improve the plan and theoretically better defined the study plan and its connotations of possible results. With contribution of GA the study was started. The data collection and input was carried out by SEO and GA. The data analysis and manuscript writing was done by SEO and the manuscript read and approved by the three authors.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate
All women gave their informed consent. The study was reviewed and approved by the Ethics Commitee of the Bagcilar Training and Research Hospital (2015/312). All patients gave informed consent for participating in this study.

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