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Assisted reproductive outcomes in women with different polycystic ovary syndrome phenotypes: the predictive value of anti-Müllerian hormone

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Abstract   This cross-sectional study aimed to evaluate IVF/intracytoplasmic sperm injection (ICSI) outcomes in different polycystic ovary syndrome (PCOS) phenotypes (A, B, C and D) compared with a control group and the predictive values of serum anti-Müllerian hormone (AMH) in PCOS phenotypes for main outcomes. This study evaluated 386 PCOS women and 350 patients with male factor infertility. Women with phenotypes A and C had significantly higher concentrations of AMH than those with phenotype B ($P < 0.001$). Clinical pregnancy rate (CPR) in the phenotype D group (53.3%) was higher than other groups (32.5%, 26.4% and 36.8%, respectively, in phenotypes A, B and C), but not to a significant level. Multivariable regression analysis, after adjusting for women's age and body mass index, revealed that PCOS phenotypes A and B were associated with a decreased CPR compared with the control group (odds ratio [OR]: 0.46, confidence interval [CI]: 0.26–0.8, $P = 0.007$ and OR: 0.34, CI: 0.18–0.62, $P = 0.001$, respectively). It seems a combination of hyperandrogenism and chronic anovulation is associated with a negative impact on the CPR in these patients. These results demonstrated that AMH concentration is related to PCO morphology but not predictive for CPR and live birth rate. RBMOnline
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Introduction

Polycystic ovary syndrome (PCOS) is a common cause of female infertility and affects 15–25% of women, based on the Rotterdam criteria (Livadas and Diamanti-Kandarakis, 2013). PCOS constitutes a continuous spectrum of symptoms starting from the early prepubertal years and continuing after menopause. Phenotypes are the clinical features resulting from the interaction between heredity and environment in a disease or syndrome (Moran et al., 2012). The phenotypic expression of PCOS varies through time and depends on several internal (e.g. genetic influence (Cui et al., 2015), ovarian/adrenal steroidogenesis and insulin resistance) and external (e.g. quality and quantity of diet, exercise and lifestyle) factors (Livadas and Diamanti-Kandarakis, 2013). Moreover, the emergence of new definitions with the use of ovarian morphology as well as chronic anovulation and hyperandrogenism, as diagnostic criteria, has developed the phenotypic variety of PCOS (Livadas and Diamanti-Kandarakis, 2013). To our knowledge, very few studies have evaluated the outcomes of assisted reproductive technology (ART) in the different phenotypes of PCOS women (Palomba et al., 2010).

Recently, anti-Müllerian hormone (AMH) has been considered a diagnostic or even prognostic marker of PCOS (Karkanaki et al., 2011). Tal et al. (2014) in a recent study stated that the concentration of serum AMH might be related to the severity of PCOS and correlate with its clinical diagnostic hallmarks (i.e. hyperandrogenism, oligo/anovulation and polycystic ovary morphology [PCOM]). Pregnancy rates are likely to decrease with the exacerbation of PCOS (Sahmay et al., 2013). Although some studies have suggested a reverse relationship between the AMH concentration and pregnancy rates (Xi et al., 2012), some others have found a positive relationship between the AMH concentration, embryo quality and clinical pregnancy rates (Tal et al., 2014).

On the other hand, an elevated concentration of basal LH due to enhanced pulsatile gonadotrophin-releasing hormone (GnRH) release is one of the hallmark endocrinological disturbances in PCOS women. Increased LH concentrations are observed in about 70% of PCOS patients with elevated LH pulse amplitude and an increased LH pulse frequency, which causes a two- to threefold elevation in serum LH concentrations versus FSH concentrations (Piouka et al., 2009). The potential impact of a high concentration of LH, and specifically a high LH/FSH ratio on human reproduction, is still under debate. Some studies reported negative impacts of high LH or LH/FSH ratio on the number of follicles as well as the number and maturity of oocytes (Tarlatzis et al., 1995), embryo quality and clinical pregnancy rates (CPR) (Wiser et al., 2013); however, other studies could not find any adverse effects (Geng et al., 2013; Mendoza et al, 2002). Elsewhere, Brodin et al., (2009) concluded that a low FSH concentration combined with high LH probably shows a well-preserved ovarian reserve and is associated with high pregnancy rates in IVF/ICSI cycles.

PCOS has its unique properties such as increased antral follicle count, serum AMH and LH/FSH ratio. Therefore, the prediction of clinical pregnancy in women with PCOS is more challenging than non-PCOS women (Sahmay et al., 2013). The present study was designed to evaluate: (i) ART outcomes in different PCOS phenotypes compared with the control group; and (ii) the predictive values of serum AMH and LH/FSH ratio in PCOS phenotypes for ART outcomes.

Materials and methods

Patients

This cross-sectional study was performed in the Royan Institute from June 2012 to January 2014. The Institutional Review Board and Ethics Committee of Royan Institute approved the study protocol on 15 June 2015 (reference number EC90/1010). The study was performed in accordance with the Helsinki Declaration and adhered to the guidelines of the Committee of Publication Ethics. All the participants signed the informed consent. All infertile women diagnosed with PCOS who underwent the first IVF/ICSI cycle were enrolled during the study period. Other causes of infertility including severe endometriosis, hydrosalpinx, uterine factor, severe male factor (oligo-tetrato-asthenozoospermia), and age factor ($\geq$40) or diminished ovarian reserve (AMH < 1 ng/ml, FSH > 12 IU/l) were excluded. Only patients with mild/moderate male factor and/or tubal factor infertility were included. Meanwhile smokers and diabetic patients were excluded from the study.

PCOS cases were diagnosed based on the Rotterdam criteria (2004), and the presence of at least two of the following criteria: menstrual irregularity (cycle length <26 days or >35 days or variation between consecutive cycles of >10 days); clinical (presence of hirsutism evaluated by a Ferriman-Gallwey score >8, severe acne and alopecia) or biochemical (total testosterone concentration >0.5 ng/ml and/or free testosterone >3.5 pg/ml) hyperandrogenism; or ultrasound evidence of polycystic ovaries. Hirsutism was assessed according to the Ferriman-Gallwey-score and examination of nine body areas for coarse terminal hair, including upper lip, chin and chest, upper and lower areas of the abdomen, thighs and upper arms. In each part, the severity of hirsutism was graded from 1 to 4 and the participants with the total score of 8 and above considered as having hirsutism. PCOM was defined as the presence of 12 or more ovarian cysts with 2–10 mm diameter per ovary and/or ovarian volume $\geq$10 cm^3. Vaginal ultrasound was performed by an ultrasound specialist and radiologist using an Aloka α-10 with a transvaginal 6–7.5 MHz probe (Medison Co., Japan). Patients with other differential diagnoses, including hyperprolactinaemia, thyroid dysfunction, hypothalamic amenorrhoea, Cushing's syndrome and ovarian failure, were detected via hormonal tests and excluded from the study. PCOS patients were categorized to four phenotype groups according to the Rotterdam criteria: (i) phenotype A: the coexistence of hyperandrogenism, chronic anovulation and polycystic ovaries (HA+AO+PCO); (ii) phenotype B: chronic anovulation and hyperandrogenism without the polycystic ovaries

(AO+HA); (iii) phenotype C: hyperandrogenism and polycystic ovaries (HA+PCO); and (iv) phenotype D: polycystic ovaries coexisting with anovulatory cycles (AO+PCO).

Normal women (who were diagnosed as male factor infertility) who underwent first IVF/ICSI cycle during the study period were considered as the control group. Male factor infertility is generally determined by two or more abnormal semen analyses, according to the World Health Organization (WHO) criteria, 2010: total sperm count $<39 \times 10^6$/ml and/or progressive motility <32% and/or normal morphology <4%.

Investigations

All the hormonal assays were performed in the Royan Institute laboratory. Blood samples were collected for the assessment of basal serum LH, FSH, AMH and fasting blood sugar (FBS) concentrations on days 2–3 of the menstrual cycle in all the women. Further evaluation in PCOS women was performed by measurement of total and free testosterone concentrations. The serum AMH concentration was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (AMH Gen II ELISA; Beckman Coulter, Inc., USA). To synchronize timing, by a specialist who performed the AMH measurements, part of the serum samples of all studied patients were collected and stored frozen at −80˚C until analysis. Finally, all AMH measurements were carried out at the end of sampling. Laboratory specialists prepared one part of each calibrator, control or test samples, respectively, with five parts of AMH Gen II assay buffer; in this preparation method, no dilution factor is required. According to kit instruction, any sample that read higher than the highest calibrator was diluted with sample dilutent, and the diluted sample was tested again. The intra-assay and inter-assay coefficients of variation were 5.4% and 5.6%, respectively. The serum concentrations of FSH and LH were measured using electro-chemiluminescence immunoassay kits (ECLIA kits, Roche Diagnostics GmbH, Germany) on an Elecsys immunoassay analyser. Total serum testosterone and free testosterone concentrations in the patients with irregular menses and/or polycystic ovaries were assessed by the ELISA kits (Monobind Inc., USA) and concentrations are given in ng/ml and pg/ml, respectively. Fasting blood sugar (FBS; mg/dl) was measured by using means of enzymatic colorimetry (Pars Azmun Co. Tehran, Iran).

Treatment

Based on the physician's decision, one of the two standard protocols of long agonist or antagonist (Stimpfel et al., 2015) was used for controlled ovarian stimulation (COH) with recombinant FSH (75–150 IU) considering the antral follicle count (AFC) and the women's age. Either IVF/ICSI or ICSI was conducted in accordance with the clinical indication. Fertilization rate was defined as the number of fertilized embryos obtained per all injected and/ or inseminated oocytes. Depending on the women's age and the quality of the embryos, two or three embryos were transferred on day 3 after oocyte retrieval. The quality of the embryos was graded as A, B, C or D (A being the best and D being the worst) based on the number of cells, degree of fragmentation and regularity.

Outcomes

All the cycle outcomes, namely freeze-all-embryos cases, fertilization, implantation, clinical pregnancy and miscarriage rates, as well as the live birth rate (LBR) were recorded. Implantation rate was defined as the number of observed intrauterine gestational sacs divided by the number of transferred embryos. A clinical pregnancy was considered as ultrasonographic visualization of an intrauterine gestational sac with fetal heartbeat. A spontaneous miscarriage was considered as a clinical pregnancy lost before 20 weeks' gestation. In PCOS patients the LH/FSH ratio was evaluated in two subgroups (LH/FSH <2.5 and LH/FSH ≥2.5), and the cut-off point was considered at 10 IU/ml for basal serum LH.

Statistical analysis

The study population's demographic factors, IVF/ICSI cycle-specific parameters and pregnancy outcomes were compared between the four PCOS phenotypes using the chi-squared test for the categorical variables and the one-way analysis of variance test (with the Tukey post-hoc test) for the continuous variables, when the data had a normal distribution, and the Kruskal-Wallis test in the non-normal cases.

In order to detect the predictive variables of clinical pregnancy rate (CPR) and LBR, the study used a multivariable logistic regression analysis in a backward manner in PCOS phenotypes and control group separately and then generally. The covariate variables in the PCOS group included: women's age and body mass index (BMI), serum AMH concentration, LH/FSH ratio, serum free testosterone and FBS concentrations, stimulation protocol, number of gonadotrophins ampoules, number of retrieved oocytes and number and quality of the transferred embryos. In the male factor (control group), covariate variables included: women's age and BMI, serum AMH concentration, infertility type, stimulation protocol, number of gonadotrophins ampoules, number of retrieved oocytes and number and quality of the transferred embryos that were entered in the multivariable logistic regression model. Finally, for multivariable logistic regression analysis adjusted for women's age and BMI in the whole study population ($n = 736$), important variables such as study groups, serum AMH concentration, LH/FSH ratio, number of retrieved oocytes, quality of transferred embryos, number of gonadotrophins ampoules and stimulation protocol were entered into the model.

It was estimated by using the NCSS software (Number Cruncher Statistical System software package 2007, Kaysville, UT, USA) that a sample size of more than 80 subjects in each group would support us to demonstrate a difference in AMH concentrations between subjects and controls of 1 ng/ml with a power of 80% and a type I error of 0.05. It was also calculated that a sample size of 350 subjects in each group would allow us to demonstrate 15% difference in CPR between subjects and controls with a power of 80% and a type I error of

0.05. The data were analysed using the Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp., USA). Statistical significance level was considered as a P-value <0.05.

Results

In total, 386 cases with a PCOS diagnosis and 350 male factor patients were enrolled during the study period. **Table 1** shows the patients' characteristics among four PCOS phenotypes and control group. The results showed that the study groups were comparable in terms of female age at cycle initiation, age at menarche, infertility type and duration of infertility. As expected, PCOS subgroups compared with the control group had significant differences in terms of BMI, serum FBS, FSH and LH concentrations. Also, AFC and serum AMH concentrations were significantly different among study groups ($P <$ 0.001). The proportions of the cases with basal LH >10 IU/l and LH/FSH ratio ≥2.5 were similar in the different phenotype groups. There were also significant differences between the phenotype groups concerning the AMH concentration insofar as phenotypes A and C had significantly higher concentrations of the serum AMH than phenotype B ($P = 0.003$ and $P = 0.01$, respectively) (**Table 1**).

Table 2 shows IVF/ICSI cycle outcomes among four PCOS phenotypes and the control group. There were no statistically significant differences among PCOS phenotypes groups in terms of stimulation duration, the total number of retrieved and metaphase II (MII) oocytes, the number and quality of the transferred embryos and endometrial thickness on the embryo transfer day. The proportions of the antagonist cycles and the freeze-all cases in the phenotype B and control group were lower than those in the other phenotype groups ($P <$ 0.001). Moreover, the phenotype B group had a tendency to have a higher number of used gonadotrophin ampoules than the other PCOS groups; although the difference was not statistically significant. The control group was significantly different in terms of stimulation protocol, number of follicles ≥12 mm, total number of embryos and the freeze-all-embryos cases compared with the four PCOS phenotypes (all $P < 0.001$).

Table 1 Comparison of the study population's characteristics among four PCOS phenotypes and control group.

	Phenotype A	Phenotype B	Phenotype C	Phenotype D	Control (Male factor)	P-value*
	(n = 168)	(n = 103)	(n = 83)	(n = 32)	(n = 350)	
Women's age (years) (mean ± SD)	32.1 ± 4.7	32.2 ± 5.3	30.8 ± 4.6	32.4 ± 4.4	31.0 ± 5.2	NS
Menarche age (years) (mean ± SD)	13.2 ± 1.5	13.4 ± 1.6	13.5 ± 1.7	12.8 ± 1.3	13.1 ± 1.5	NS
BMI (kg/m²) (mean ± SD)	27.6 ± 3.9[e]	27.5 ± 4.9[e]	27.1 ± 3.9[e]	26.4 ± 4.0	25.2 ± 4.0	<0.001
FSH (IU/l) (mean ± SD)	5.8 ± 1.9[e]	5.9 ± 2.6[e]	5.8 ± 2.2[e]	6.2 ± 2.7	6.9 ± 3.1	<0.001
LH (IU/l) (mean ± SD)	8.4 ± 5.7[e]	6.9 ± 4.8[e]	7.5 ± 5.4[e]	7.7 ± 6.4[e]	4.9 ± 3.2	<0.001
AMH (ng/ml) (mean ± SD)	6.8 ± 2.8[b,e]	5.1 ± 3.1[a,c,e]	6.3 ± 2.9[b,e]	5.8 ± 2.4[e]	2.3 ± 1.8	<0.001
FBS (mg/dl) (mean ± SD)	89 ± 12.6[b,e]	88.0 ± 10.2[a,c,d]	91.0 ± 10.5[b,e]	91.1 ± 8.9[b,e]	86.7 ± 6.4	<0.001
Total testosterone concentration (ng/ml) (mean ± SD)	1.3 ± 0.6[c,d]	1.1 ± 0.5[c,d]	1.0 ± 0.3[a,d]	0.3 ± 0.1[a,b,c]	–[#]	<0.001
Free testosterone concentration (pg/ml) (mean ± SD)	4.1 ± 0.9[c,d]	3.9 ± 0.8[c,d]	3.7 ± 0.4[a,b,c]	1.8 ± 0.7[a,b,c]	–[#]	<0.001
Basal LH >10 IU/l, n (%)	49 (29.2)	23 (22.3)	21 (25.3)	9 (28.1)	22 (6.3)	<0.001
LH/FSH ratio ≥2.5, n (%)	25 (14.9)	18 (17.5)	10 (12.0)	4 (12.5)	9 (2.6)	<0.001
AFC (mean ± SD)	35.4 ± 4.8[b,d,e]	20.0 ± 1.8[a,c,d,e]	35.1 ± 4.6[b,d,e]	33.2 ± 3.9[a,b,c,e]	11.2 ± 2.4	<0.001
Type of infertility, n (%)						NS
Primary	146 (87.4)	90 (87.4)	75 (90.4)	28 (87.5)	326 (93.1)	
Secondary	21 (12.6)	13 (12.6)	8 (9.6)	4 (12.5)	24 (6.9)	
Cause of infertility, n (%)						NS
PCOS	98 (58.7)	62 (60.2)	46 (55.4)	19 (59.4)	–	
PCOS and male factor	69 (41.3)	41 (39.8)	37 (44.6)	13 (40.6)	–	
Infertility duration (years)	6.8 ± 2.9	6.9 ± 2.8	6.2 ± 2.9	6.2 ± 2.5	6.8 ± 3.8	NS

AFC = antral follicle count; BMI = body mass index; FBS = fasting blood sugar; NS = not statistically significant; PCOS = polycystic ovary syndrome.
[a]Significantly different from phenotype A (the coexistence of hyperandrogenism (HA), chronic anovulation (AO), and polycystic ovaries).
[b]Significantly different from phenotype B (HA and AO without the polycystic ovaries).
[c]Significantly different from phenotype C (HA and polycystic ovaries).
[d]Significantly different from phenotype D (AO and polycystic ovaries).
[e]Significantly different from control group.
*All P-values for quantitative variables were determined by post-hoc analysis (Tukey).
[#]Serum testosterone levels were measured in patients with irregular menses or clinical signs of hyperandrogenism and/or PCOS morphology in sonography.

Table 2 Comparison of cycle outcomes among four PCOS phenotypes and control group.

	Phenotype A	Phenotype B	Phenotype C	Phenotype D	Control group (Male factor)	*P-value
	(n = 168)	(n = 103)	(n = 83)	(n = 32)	(n = 350)	
Stimulation protocol, n (%)						
Long agonist	79 (47) [e]	79 (76.7)[a,c,d,e]	39 (47)[e]	17 (53.1)[e]	318 (90.9)	<0.001
Antagonist	89 (53)	24 (23.3)	44 (53)	15 (46.9)	32 (9.1)	
No. of gonadotropin ampoules (mean ± SD)	22.1 ± 11.8[e]	25.3 ± 9.8	22.7 ± 7.3[e]	23.3 ± 7.2	26.8 ± 9.8	<0.001
Stimulation duration (days) (mean ± SD)	10.1 ± 2.3	10.4 ± 2.1	10.3 ± 1.9	10.3 ± 2.1	10.8 ± 2.1	NS
No. of follicles ≥ 12 mm (mean ± SD)	15.6 ± 11.0[b,e]	12.4 ± 5.0[e]	14.1 ± 7.5[e]	12.7 ± 8.7[a,e]	12.1 ± 6.5	<0.001
No. of retrieved oocytes (mean ± SD)	11.3 ± 6.7	11.0 ± 7.3	11.2 ± 6.3	11.2 ± 7.1	10.1 ± 5.5	NS
No. of MII oocytes (mean ± SD)	9.2 ± 4.7	9.2 ± 5.9	9.2 ± 5.1	9.5 ± 6.2	8.6 ± 5.4	NS
Total no. of embryos (mean ± SD)	7.7 ± 4.5[e]	7.2 ± 4.6[e]	7.0 ± 4.6[e]	7.6 ± 4.4[e]	5.5 ± 4.1	<0.001
No. of transferred embryos (mean ± SD)	2.2 ± 0.6	2.3 ± ± 0.7	2.1 ± 0.6	2.2 ± 0.5	2.3 ± 0.7	NS
Quality of transferred embryos, n (%)						NS
Good (quality of all transferred embryos was A, B, and AB)	60 (77.9)	55 (76.4)	25 (65.8)	10 (66.7)	176 (63.5)	
Fair (half of transferred embryos were good quality (AC and BC)	12 (15.6)	15 (20.8)	10 (26.3)	4 (26.7)	76 (27.4)	
Poor (quality of all transferred embryos was poor (C and CD)	5 (6.5)	2 (2.8)	3 (7.9)	1 (6.7)	25 (9.0)	
Endometrial thickness on ET day (mean ± SD)	9.9 ± 1.3	9.8 ± ± 1.6	9.6 ± 1.6	9.9 ± 1.2	9.8 ± 1.7	NS
Freeze-all-embryos	83 (49.4)[e]	28 (27.2)[a,c,d,e]	40 (48.2)[e]	16 (50.0)[e]	40 (11.4)	<0.001
Cancellation rate (no oocyte, no embryo and abnormal endometrial thickness)	8 (4.8)	3 (2.9)	5 (6.0)	1 (3.1)	35 (10.0)	NS
Fertilization rate (mean ± SD)	0.75 ± 0.22[e]	0.74 ± 0.23[e]	0.77 ± 0.21[e]	0.76 ± 0.22[e]	0.64 ± 0.26	<0.001
Implantation rate (mean ± SD)	0.19 ± 0.31[e]	0.20 ± 0.33[e]	0.23 ± 0.32[e]	0.31 ± 0.32	0.49 ± 0.28	<0.001
Clinical pregnancy rate/ET (%)	25/77 (32.5)	19/72 (26.4)	14/38 (36.8)	8/15 (53.3)	124/275 (45.1)	0.02
Multiple pregnancy rate n (%)	6 (24)	6 (31.6)	5 (35.7)	1 (12.5)	29/124 (23.4)	NS
Miscarriage rate/ET (%)	4/77 (5.2)	0/72 (0)	3/38 (7.9)	1/15 (6.7)	31/275 (11.3)	NS
Live birth rate/ET (%)	21/77 (27.3)	19/72 (26.4)	11/38 (28.9)	7/15 (46.7)	93/275 (33.8)	NS

ET = embryo transfer; MII = metaphase II; NS = not statistically significant; PCOS = polycystic ovary syndrome.
[a] Significantly different from phenotype A (the coexistence of hyperandrogenism (HA), chronic anovulation (AO), and polycystic ovaries).
[b] Significantly different from phenotype B (HA and AO without the polycystic ovaries).
[c] Significantly different from phenotype C (HA and polycystic ovaries).
[d] Significantly different from phenotype D (AO and polycystic ovaries).
[e] Significantly different from control group.
*All P-values for quantitative variables were determined by post-hoc analysis (Tukey).

Although fertilization rate was significantly lower in the control group (P < 0.001), the implantation rate and CPR was significantly higher than PCOS phenotypes A, B and C (P < 0.001). However, miscarriage and LBR were not significantly different among groups. A tendency to higher implantation rate, CPR and LBR was observed in patients with phenotype D compared with the other PCOS phenotypes; however, these differences did not reach significant levels (Table 2).

The study compared the main outcomes between two groups based on the presence of the PCOM by vaginal ultrasonography (Table 3). The results showed that women with PCOM had significantly higher concentrations of serum AMH (P = 0.001) and there was a non-significant trend towards higher LH values in the PCOM group. The rate of freeze-all-embryos was greater in PCOS women with the presence of the PCOM in ultrasonography (P = 0.001). No relationship between

Table 3 Comparison of the main outcomes between two groups based on the observation of the PCO morphology by vaginal ultrasonography.

Variables	Women with PCO morphology (Phenotypes A, C, and D) (n = 283)	Women without PCO morphology (Phenotype B) (n = 103)	P-value
AMH (ng/ml) (mean ± SD)	6.3 ± 2.8	5.1 ± 3.1	0.001
LH (IU/l) (mean ± SD)	8.1 ± 5.7	6.9 ± 4.8	0.05
LH/FSH ratio (mean ± SD)	1.5 ± 1.2	1.4 ± 1.0	NS
Freeze-all-embryos, n (%)	139 (49.1)	28 (27.2)	0.001
Clinical pregnancy rate/ET (%)	47/130 (36.2)	19/72 (26.4)	NS
Live birth rate/ET (%)	39/130 (30)	19/72 (26.4)	NS

AMH = anti-Müllerian hormone; ET = embryo transfer; PCO = polycystic ovarian; MII = metaphase II; NS = not statistically significant.

Table 4 Multivariable logistic regression analysis by backward manner for determination of the predictive variables for clinical pregnancy and live birth rates in PCOS phenotypes and control (male factor) groups separately.

Variables		Predictive factors	OR	95% CI	P-value
PCOS Phenotype A (n = 168)	Clinical pregnancy and live birth	Serum free testosterone concentration	0.4	(0.3–0.75)	0.01
PCOS Phenotype B (n = 103)	Clinical pregnancy and live birth	Serum AMH concentration	1.3	(1.05–2.0)	0.01
		Serum free testosterone concentration	0.5	(0.4–0.9)	0.02
PCOS Phenotype C (n = 83)	Clinical pregnancy and live birth	Women's age	0.8	(0.6–-1.02)	NS
PCOS Phenotype D (n = 32)	Clinical pregnancy and live birth	Women's age	0.8	(0.6–1.07)	NS
All PCOS women (n = 386)	Clinical pregnancy	Serum free testosterone concentration	0.6	(0.1–0.8)	0.02
		Quality of transferred embryos			
		Good quality (A, B, and AB)	Reference group		
		Fair quality (AC and BC)	0.47	(0.1–2.2)	NS
		Poor quality (C and CD)	0.34	(0.13–0.86)	0.02
	Live birth	Women's age	0.9	(0.85–0.99)	0.04
		Serum free testosterone concentration	0.5	(0.12–0.85)	0.01
		Quality of transferred embryos			
		Good quality (A, B, and AB)	Reference group		
		Fair quality (AC and BC)	0.3	(0.05–1.7)	NS
		Poor quality (C and CD)	0.4	(0.15–1.006)	NS
Male factor (control group) (n = 350)	Clinical pregnancy	Total number of retrieved oocytes	1.1	(1.01–1.2)	0.01
		Quality of transferred embryos			
		Good quality (A, B, and AB)	Reference group		
		Fair quality (AC and BC)	0.6	(0.3–1.1)	0.1
		Poor quality (C and CD)	0.1	(0.02–0.38)	0.001
	Live birth	Total number of retrieved oocytes	1.06	(1.002–1.2)	0.04
		Quality of transferred embryos			
		Good quality (A, B, and AB)	Reference group		
		Fair quality (AC and BC)	0.5	(0.3–1.1)	NS
		Poor quality (C and CD)	0.1	(0.01–0.5)	0.009

AMH = anti-Müllerian hormone; CI = confidence interval; OR = odds ratio; NS = not statistically significant; PCO = polycystic ovarian.

the presence of the PCOM and the LH/FSH ratio, CPR or LBR was found.

A multivariable logistic regression analysis by backward manner adjusted for women's age and BMI was performed in each PCOS phenotype separately (Table 4). The results demonstrated that in PCOS phenotypes A and B, serum free testosterone concentration was the significant predictor for CPR and LBR (odds ratio [OR]: 0.4, confidence interval [CI]: 0.3–0.75; P = 0.01; OR: 0.5, CI: 0.4–0.9; P = 0.02, respectively).

This means that with every 1 pg/ml increase in serum free testosterone concentration, CPR and LBR decrease 50–60% in these patients. In addition, in women with PCOS phenotype B, serum AMH concentration was the significant predictor for CPR and LBR (OR: 1.3, CI: 1.05–2.0; P = 0.01); in which for every 1 ng/ml increase in serum AMH concentration, the chance of clinical pregnancy and live birth increase 1.3 times. The analysis in other PCOS phenotypes (C and D) showed that only women's age was important for prediction of CPR and

Table 5 Multivariable logistic regression analysis by backward manner for determination of the predictive variables for clinical pregnancy and live birth rates in whole study population, adjusted for women's age and body mass index ($n = 736$).

Variables	Predictive factors	Adjusted OR	CI	P-value
Clinical pregnancy	Total number of retrieved oocytes	0.90	(0.81–0.97)	0.03
	Quality of transferred embryos			
	Good quality (A, B, and AB)	Reference group		
	Fair quality (AC and BC)	0.52	(0.33–0.84)	0.008
	Poor quality (C and CD)	0.17	(0.1–0.4)	0.001
	Study groups			
	PCOS phenotype A	0.46	(0.26–0.8)	0.007
	PCOS phenotype B	0.34	(0.18–0.62)	0.001
	PCOS phenotype C	0.6	(0.3–1.2)	NS
	PCOS phenotype D	2.0	(0.62–6.6)	NS
	Male factor (control group)	Reference group		
Live birth	Total number of retrieved oocytes	0.9	(0.80–0.99)	0.04
	Quality of transferred embryos			
	Good quality (A, B, and AB)	Reference group		
	Fair quality (AC and BC)	0.57	(0.35–0.93)	0.02
	Poor quality (C and CD)	0.15	(0.1–0.5)	0.003
	Study groups			
	PCOS phenotype A	0.59	(0.33–1.06)	NS
	PCOS phenotype B	0.57	(0.31–1.03)	NS
	PCOS phenotype C	0.7	(0.33–1.5)	NS
	PCOS phenotype D	2.3	(0.75–7.5)	NS
	Male factor (control group)	Reference group		

CI = confidence interval; NS = not statistically significant; OR = odds ratio; Phenotype A = the coexistence of hyperandrogenism (HA), chronic anovulation (AO), and polycystic ovaries); Phenotype B = the coexistence of HA and AO without the polycystic ovaries; Phenotype C = the coexistence of HA and polycystic ovaries; Phenotype D = the coexistence of AO and polycystic ovaries; PCOS = polycystic ovarian syndrome.

LBR; however, it did not reach a significant level, probably owing to low sample sizes in these two subgroups (Table 4).

Multivariable logistic regression analysis by backward manner was performed in the PCOS group generally ($n = 386$). The analysis showed that serum free testosterone concentration and quality of transferred embryos were important predictors for CPR in PCOS women ($P = 0.02$). It also demonstrated that women's age and serum free testosterone concentration were the significant predictors for LBR in these women ($P = 0.04$ and $P = 0.01$, respectively). It means that in PCOS women, with every one-year increase in women's age the LBR reduces by 10%. Also, with every 1 pg/ml increase in serum free testosterone concentration, the LBR decreases by 50%. The poor quality of transferred embryos was an important predictive variable for LBR; however, it did not reach a significant level (OR: 0.6, CI: 0.015–1.006) (Table 4).

In a similar way, the control group was evaluated by multivariable logistic regression analysis by backward manner to find the important predictive variables for CPR and LBR in this population. Results revealed that the total number of retrieved oocytes and quality of transferred embryos were the significant predictors ($P = 0.01$ and $P = 0.001$, respectively). It means that in women with male factor infertility, with every increase of one in the total number of retrieved oocytes, the chance of CPR increases 1.1 times. Also, the CPR and LBR in women with poor quality of transferred embryos decreased by 90% (Table 4).

The result of the backward multivariable logistic regression analysis in the whole study population after adjusting for women's age and BMI ($n = 736$) indicated that in addition to the total number of retrieved oocytes and quality of transferred embryos, the PCOS phenotype A and B were significant predictive factors for CPR and LBR. This means that these phenotypes were associated with a decreased CPR compared with the male factor group (OR: 0.46, CI: 0.26–0.8, $P = 0.007$ and OR: 0.34, CI: 0.18–0.62, $P = 0.001$, respectively) (Table 5).

Discussion

The present study compared the IVF/ICSI outcomes in four phenotypes of PCOS women and the control group and also evaluated the predictive values of the LH/FSH ratio and AMH concentration for CPR and LBR. The IVF/ICSI outcomes were similar between the different PCOS phenotypes, but PCOS phenotypes A and B were associated with a 54% and 66% decrease in CPR compared with the control group, respectively. It seems that a combination of hyperandrogenism and chronic anovulation in patients with phenotypes A and B had a negative effect on CPR and LBR. Similarly, Palomba et al. (2010), evaluated the effect of different PCOS phenotypes on obstetric and neonatal outcomes and concluded that the risk of adverse obstetric and neonatal outcomes in PCOS women varies widely according to the different phenotypes and features of PCOS. Their results showed that the risk of adverse obstetric or neonatal outcomes was affected significantly by ovarian dysfunction and biochemical hyperandrogenism,

whereas no significant effect was detected for clinical hyperandrogenism and the PCOM (Palomba et al., 2010).

Results from this study demonstrated a relationship between the serum AMH concentration and PCOS phenotypes, that the phenotype B women had significantly lower concentrations of AMH than the women with phenotypes A and C. These findings are in agreement with previous studies reporting that the serum AMH concentration is allied to the severity of PCOS (Tal et al., 2014). Likewise, Parahuleva et al. (2014), reported some differences in the values of AMH in the four main PCOS phenotypes and found direct correlations between the concentrations of AMH and other hormonal parameters. Based on the recent studies (Casadei et al., 2013; Eilertsen et al., 2012; Iliodromiti et al., 2013) highlighting differences in serum AMH concentrations in various phenotypic expressions of PCOS, the AMH concentration may serve as a reliable tool for the prediction, diagnosis, monitoring and categorization of the severity of this syndrome. In this regard, Dewailly et al. (2011), concluded that a serum AMH >5 ng/ml seems to be more sensitive and specific than follicle number in the diagnosis of PCOM; therefore it should be considered in current diagnostic classification for PCOS. Along the same lines, Casadei et al. (2013), suggested that the measurement of AMH may be considered as the main diagnostic criterion for the diagnosis of PCOS when either hyperandrogenism or anovulation is missing and/or when no reliable AFC can be measured.

This study also found that AMH concentration was predictive for CPR and LBR only in PCOS women with phenotype B, whereas it was not predictive in other PCOS phenotypes. In this regard, Tal et al. (2014), showed that PCOS women with ultra-high AMH concentrations had significantly more good-quality embryos and increased CPR. Conversely, Xi et al. (2012), reported that CPR was lower in PCOS women with high concentrations of AMH, as the clinical pregnancy rates were 65%, 66.7% and 45.9%, in <25%, 25–75% and >75% percentiles of the serum AMH groups, respectively. Elsewhere, Sahmay et al. (2013), demonstrated that the mean AMH concentrations were not significantly different between pregnant and non-pregnant PCOS women. In their study, CPR increased in parallel with a rise in the AMH percentiles, although this relationship was insignificant. Only a few studies have evaluated the predictive value of serum AMH for pregnancy in a PCOS-only group (Sahmay et al., 2013; Tal et al., 2014; Xi et al., 2012). The conflicting results of these studies can be due to the differences in measurement methods and categorization of AMH concentrations as well as the presence of various PCOS phenotypes in different races (Sahmay et al., 2013). It seems that more studies are necessary to draw a definitive conclusion regarding the predictive value of serum AMH for ART outcomes in PCOS-only patients.

Results from this study showed that in women with male factor infertility, total number of retrieved oocytes and quality of transferred embryos were significant predictors for CPR and LBR, whereas serum AMH concentration was not a significant predictor. Previous studies reported that AMH is a significant predictor for CPR (Elgindy et al., 2008; Hazout et al., 2004; Lekamge et al., 2007; Wu et al., 2009), while some others could not find such results (Choi et al., 2011; Penarrubia et al., 2005; Smeenk et al., 2007). Two recent prospective studies reported the predictive value of AMH for LBR (Lee et al., 2009; Nelson et al., 2007). Nelson et al. (2007), reported

that LBR increased with increasing AMH concentrations; although this is significant only for patients with basal AMH concentrations <7.8 pmol/l and in patients with AMH above this value, there was no relationship between live birth and AMH. However, in their study, after multivariable regression analysis, they found that oocyte yield was the only significant predictive variable for live birth. Elsewhere, Wang et al. (2010), concluded that AMH concentration is an important factor to predict IVF pregnancy rates for women aged between 34 and 41 years; however, it has limited predictive value in younger and older women. In a recent study, Khader et al. (2013), confirmed the external validity of a particular nomogram to determine the probability of live births by age and AMH; however, Mutlu et al. (2013), in a recent prospective study, reported that female age is the only predictor for LBR in IVF cycles. Similarly, Broer et al. (2013), in a recent meta-analysis reported that the best single predictor for ongoing pregnancy was women's age. According to the conflicting results, it seems that the role of infertility aetiology must be important in evaluating the predictive values of different variables for ART outcomes. Presumably, in different infertility subgroups, the variables affecting the probability of pregnancy and live birth have different predictive values.

This study's results indicate that high basal LH concentrations (≥10 IU/l) and high LH/FSH ratios (≥2.5) had no negative effect on CPR and LBR in IVF/ICSI cycles in PCOS women. In this regard, Geng et al. (2013), evaluated 134 PCOS women retrospectively and concluded that a basal LH concentration >10 IU/l or a high LH/FSH ratio (≥2) did not produce an obvious deleterious impact on the clinical results of IVF-embryo transfer in their PCOS women, who were on oral contraceptives for pretreatment before the long GnRH-agonist protocol. On the other hand, Wiser et al. (2013), reported that possibly the high basal LH concentration impaired ovarian folliculogenesis, oocyte quality and consequently the pregnancy rate in in-vitro maturation cycles. The fact that the ovarian stimulation protocol in our study population was a standard agonist or antagonist seems to confirm the finding of the study by Tarlatzis et al. (1995), who remarked that using the GnRH agonist or antagonist in the standard protocol overcame the possible negative effect of elevated basal LH on follicular and oocyte development.

The limitation of this study was the absence of a uniform COH protocol in all the patients. Nonetheless, the effect of the COH protocol on the main outcomes was evaluated using a multivariable regression test that demonstrated that the COH protocol had no impact on CPR and LBR.

In conclusion, this study found that in PCOS women a combination of hyperandrogenism and chronic anovulation as in phenotypes A and B were associated with lower CPR and LBR compared with the control group. The study suggested that the serum AMH concentration was related to PCOM but is not predictive for CPR and LBR. However, further prospective studies are needed to confirm or refute these findings.

Acknowledgements

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