Ovarian Endocrine Status and ART Outcomes in Women within PCOS Based on Different Testosterone Levels

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Abstract

Background: It is estimated that in women at reproductive age, the risk of polycystic ovary syndrome (PCOS) is about 5–21%. In PCOS cases with ovulation dysfunction, assisted reproductive techniques (ART) are useful for infertility treatment. **Objective:** This study aimed to evaluate the ART outcome in infertile PCOS women based on different testosterone levels. Finally, the relationships between testosterone in different levels and reproductive parameters including endocrine status, the response of ovaries, and pregnancy outcomes were assessed. **Methods:** In this retrospective study, 352 infertile PCOS women were examined. The women were categorised into five groups according to their testosterone levels: A = T < 0.4, B = 0.4 < T > 0.6, C = 0.6 < T > 0.8, D = 0.8 < T > 1.0 and E = T > 1.0 ng/dL. All study cases were in similar hyper-stimulation protocol and finally, hormonal profile and ART outcomes were compared between testosterone levels. P value ≤ 0.05 was statistically significant. **Results:** In testosterone levels >1.0, the levels of anti-mullerian hormone (AMH) and luteinising hormone (LH) were higher than in other testosterone level groups. AMH ($P = 0.05$) and LH ($P = 0.001$) levels showed significant differences. No correlation was present between testosterone levels and ART outcomes, including stimulation duration, endometrial thickness, oocyte numbers, numbers of matured oocytes, number of obtained embryos, fertilisation rate, implantation rate clinical pregnancy and abortion rate. **Conclusions:** Serum testosterone levels did not show any correlation with pregnancy outcomes in ART cycles of PCOS. However, basal testosterone levels are a good predictor for ovarian reserve and ovarian response. Consequently, we suggest that some prospective studies must be designed to approve the role of testosterone in the prediction of the outcome of pregnancy in ART cycles.

Keywords: Assisted reproductive techniques (ART), polycystic ovary syndrome (PCOS), pregnancy, Rotterdam criteria, testosterone

Background

Polycystic ovary syndrome (PCOS) is a condition that can affect some systems of the human body including the endocrine and reproductive system. Also, PCOS has a negative effect on female fertility and about 5–21% of women who are of reproductive age suffer from this fertility problem. PCOS has different symptoms including ovulation dysfunction, defect in the menstrual cycle and hirsutism.[1] Metabolic syndromes with hyperandrogenemia, dyslipidaemia, and insulin resistance are some characteristics of PCOS.[2]

Numerous studies focused on PCOS criteria. There was an idea that hyperandrogenism is the main key in PCOS. This idea is accepted by different medical researchers. However, there is no agreement on the classification of hyperandrogenism. Some documents believe that in a different population,

hirsutism in visual scales is the main clinical parameter of hyperandrogenism.[3]

Laboratory indications of hyperandrogenaemia are unjustifiable increase in serum androgenic components such as serum testosterone levels and free androgen index (FAI). This elevation in androgen is frequently due to PCOS and can create insulin resistance in a female's body.[4] This point that hyperandrogenaemia is an important factor in the

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creation of insulin resistance has been reported by several researchers.[5,6]

The role of androgens as the creator of oestrogens is important in regular ovarian function such as the development of follicular maturation and the progress of the ovulation cycle.[7] This point can open a new window in the future for the treatment of ovulation disorders by medications based on androgens. However, to date, detailed mechanisms of androgen in the maturation of follicles remain unknown and the scientific documents in this regard are controversial.[8]

Also, evaluation of the effects of increased androgen levels in PCOS on other hormones showed that in these patients, an imbalance occurred in some hormones such as anti-mullerian hormone (AMH), follicle-stimulating hormone (FSH), luteinising hormone (LH) and oestradiol (E2). In PCOS patients, the serum level of AMH was higher than in normal cases. In infertile PCOS women, a high number of small-antral follicles was observed in sonography. Studies suggested that higher serum AMH was correlated with the number of small-antral follicles; however, the correlation between the polycystic pattern in PCOS cases and the level of serum androgen is controversial.[9]

Objective

In infertile PCOS cases with ovulation dysfunction, assisted reproductive techniques (ART) are useful for ovulation induction and infertility treatment.[10] Thus, this study aimed to evaluate the ART outcome in infertile PCOS women based on different testosterone levels. Finally, the relationships between testosterone in different levels and reproductive parameters including endocrine status, the response of ovaries and pregnancy outcomes were assessed.

Methods

The present study was a retrospective cohort study and was approved by the Ethics Committee of Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran by ethical code (IR.SSU.RSI. REC.1399.001). In this study, we reviewed the clinical and laboratory data of women at the Gynaecology Cinic at Yazd Research and Clinical Centre for Infertility. All PCOS women who were in their first ART cycle and treated using the GnRH antagonist protocol to control ovarian hyper-stimulation were included in the study from April 2015 to August 2017. Women's age was between 18 and 40 years and the diagnosis of PCOS was based on the Rotterdam criteria.[11] Patients with the presence of the severe male factor, severe endometriosis, hydrosalpinx and history of any endocrine disorder, except PCOS, were excluded from the study. The women were categorized into five groups according to their testosterone levels: A = T < 0.4, B = 0.4 < T > 0.6, C = 0.6 < T > 0.8, D = 0.8 < T > 1.0 and E = T > 1.0 ng/dL based on the Xiao method.[12] All women were in similar hyper-stimulation protocol and triggered after observation of at least two

follicles (17 mm in diameter). Oocyte retrieval was performed under general anaesthesia after 36 h transvaginally. Oocytes are fertilised using two laboratory methods including *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI), when appropriate. All formed embryos were evaluated 2 days later morphologically. In PCOS patients based on the clinical plan, transfer of fresh embryos was cancelled; therefore, all high-quality embryos with fragmentations lower than 30% were cryopreserved using the same freeze method 2 days after ovarian puncture. All frozen-thawed procedures are performed according to Vitrolife guidelines. Two months later, uterine synchronisation was performed for all cases by a similar protocol and embryo transfer was carried out after towing embryos. Patient data were collected from hospital records and included age as well as clinical and laboratory features of ART cycles. Chemical pregnancy was measured by β-HCG 2 weeks after embryo transfer and clinical pregnancy by observing the foetal heart rate 2–3 weeks after the positive pregnancy test by ultrasound. The abortion rate was defined as pregnancy losses earlier than 20 weeks of gestation per positive chemical pregnancy. The implantation rate was measured by dividing the number of gestational sacs by the number of transferred embryos. The Fertilization rate was measured as the number of two pronuclei (2PN) divided by the number of metaphase II (MII) oocytes and implantation rate was calculated as the number of gestational sacs divided by the number of transferred embryos.

Statistical analysis
All data were analysed by SPSS version 20 (IBM, Armonk, NY, USA). P values under 0.05 were considered statistically significant. Results were presented as mean ± standard deviation (SD) for quantitative and percentage for qualitative parameters.

Results

The patients who were finally analysed for this study were 356 women. The mean age at the start of the ART was 28.34 ± 4.89 years. The mean body mass index (BMI) was 28.86 ± 3.43 kg/m². Table 1 shows the mean demographic, hormonal and biochemical parameters. Vitamin D3 and fasting blood sugar were 21.31±9.18 (ng/mL) and 97.90 ± 16.21 (mg/dL) respectively [Table 1].

We also analysed hormonal parameters based on testosterone levels (T < 0.4, 0.4 < T > 0.6, 0.6 < T > 0.8, 0.8 < T > 1.0 and T > 1.0 ng/dL) and reported in Table 2. In testosterone levels >1.0, the levels of AMH and LH were higher than in other testosterone level groups. There was a significant difference in AMH (P = 0.05) and LH (P = 0.001) levels [Table 2].

Table 3 shows the controlled ovarian hyperstimulation (COH) parameters in different testosterone levels. There was no correlation between testosterone levels and ART outcome. Stimulation duration, endometrial thickness, oocyte numbers, number of matured oocytes, number of obtained embryos, fertilisation rate, implantation rate, clinical pregnancy and

Table 1: Demographic, hormonal and biochemical parameters in all study cases

parameters	Patients *n* = 352
Age (years)	28.34±4.89
BMI (kg/m²)	28.86±3.43
Infertility duration (years)	5.69±3.73
Infertility type	
Primary *n* (%)	269 (76.4)
Secondary *n* (%)	83 (23.6)
PCO phenotype	
A *n* (%)	127 (36.3)
B *n* (%)	55 (15.7)
C *n* (%)	67 (18.9)
D *n* (%)	103 (29.1)
AMH	9.42±4.20
E2 (pg/mL)	3942.37±2345.38
FSH (IU/mL)	5.51±2.29
LH (IU/mL)	9.75±5.84
Testosterone (ng/dL)	0.86±0.48
FBS	97.90±16.21
Vitamin D3	21.31±9.18
Gonadotropin dose (IU)	1565.96±774.16
Stimulation days	9.63±1.75
Endometrial thickness (mm)	9.26±1.07

Note: AMH=Anti-mullerian Hormone, E2=Estradiol, LH=Luteinising hormone; FSH=Follicle-stimulating hormone, FBS=Fasting blood sugar

abortion rate were not statistically significantly different between the five testosterone level groups.

DISCUSSION

The aim of the present study was to evaluate the correlation between serum testosterone levels with ovarian endocrine status and ART outcome in infertile PCOS women. The results of the present study showed that there was a positive correlation between testosterone and AMH levels. To date, there has been no definite opinion among researchers about the role of androgens in follicular development and ovulation.[8] The results of the present study cannot show any positive or negative relationship between different testosterone levels in the early follicular phase, follicular growth parameters, and finally pregnancy rate. According to our results, we did not find any correlation between testosterone levels and ART outcome, and there was no statistically significant difference between testosterone levels and stimulation duration, endometrial thickness, oocyte numbers, number of matured oocytes, number of obtained embryos, fertilisation rate, implantation rate, clinical pregnancy and abortion rate.

Fiza *et al.* in their study showed the ratio between two main parameters including level of serum anti-mullerian hormone and both luteinizing hormone/follicle stimulating hormone and reported the level of sex hormones and AMH were higher in PCOS woman. They concluded that increased serum testosterone is associated with an additional increase in anti-mullerian hormone, luteinising hormone, estradiol, dehydroepiandrosterone and LH/FSH ratio, whereas serum FSH showed a decreasing pattern that further justifies the increase in the LH/FSH ratio.[9]

According to our review of the literature, we did not find any study that evaluated the relationship between testosterone levels and ART outcome in PCO women.

Shan *et al.*, in a retrospective study, investigated the effects of serum testosterone in basal levels on IVF outcomes in women under fertility treatment protocol. They selected 495 women with regular menstruation and before enrolment in the IVF cycle, the level of serum testosterone was measured. They reported that in cycling women, levels of basal serum testosterone were negatively related to the age of women and FSH/LH ratios. They also found that with increased serum testosterone levels, the number of oocytes and embryo formation was higher than with low levels of serum testosterone; however, there was no statistically significant relationship between basal serum testosterone levels and clinical pregnancy rate. Finally, they concluded that basal serum testosterone levels were significantly related to main ovarian reserve parameters including age and FSH/LH ratios, and high levels of basal serum testosterone did not have positive effects on the outcome of pregnancy in cycling women under IVF treatment.[12]

Also, in other retrospective studies, Sun *et al.*, in 2014, measured basal testosterone levels in infertile women who were candidates for IVF to evaluate the relationship between basal testosterone levels and IVF parameters. Basal testosterone levels were evaluated for 1,413 infertile women whose cause of infertility was not PCOS and endometriosis. All patients were in similar IVF treatment protocols and based on basal testosterone levels, they were divided into two groups; Group 1, basal testosterone levels lower than 20 ng/dL (*n* = 473), and Group 2, basal testosterone levels higher than 20 ng/dL (*n* = 940). They reported study that parameters including BMI, basal FSH levels, basal LH levels, antral follicle count (AFC), stimulation days, total gonadotrophin dose, basal FSH/LH ratio and the number of dominant follicles >14 mm were significantly different between the two groups. Also, they reported a correlation between basal testosterone levels and ovarian reserve, the total dosage of gonadotropin and the total number of dominant follicles on human chorionic gonadotrophin (HCG) day. Finally, they concluded that basal testosterone levels are a useful parameter for the prediction of ovarian response and the number of dominant follicles on HCG day and is a good marker for calculating FSH dosage. However, basal T levels do not predict pregnancy outcomes in infertile women undergoing IVF treatment protocol.[13]

To date, the predicting value of basal testosterone levels on pregnancy outcome in IVF cycles is controversial. Walters *et al.*,[14] in 2010, measured the threshold levels and confirmed that the basal testosterone levels on day 3 in IVF cycles were a useful parameter to predict the success rate of pregnancy

Table 2: Comparison of hormonal parameters based on testosterone levels

Parameters	T < 0.4 $n=61$	0.4 < T >0.6 $n=45$	0.6 < T > 0.8 $n=63$	0.8 < T > 1.0 $n=50$	T > 1.0 $n=133$	P
AMH	9.11±3.89	8.87±4.87	9.84±5.15	8.14±3.17	10.04±3.84	0.05
E2	3734.43±2603.56	3818.47±2245.44	3924.38±2435.51	3346.78±2131.96	4312.10±2257.10	0.12
LH	8.22±5.30	9.14±5.25	8.64±5.53	9.85±5.35	11.20±6.31	0.001
FSH	5.47±2.24	5.55±1.89	5.56±2.29	5.57±2.11	5.47±2.51	0.99

Data extracted by one-way ANOVA test. $P \leq 0.05$ was considered statistically significant. Note: AMH: Anti-mullerian Hormone; E2: Estradiol; LH: Luteinising hormone; FSH: Follicle-stimulating hormone

Table 3: Comparison of COH and ART outcomes based on testosterone levels

Parameters	T <0.4 $n=61$	0.4 < T > 0.6 $n=45$	0.6 < T > 0.8 $n=63$	0.8 < T > 1.0 $n=50$	T > 1.0 $n=133$	P
Stimulation duration	9.80±1.75	9.71±2.09	9.52±1.76	9.76±2.02	9.53±1.51	0.800
Endometrial thickness	9.25±0.96	9.35±1.24	9.37±1.30	9.21±0.97	9.20±0.97	0.820
Oocyte, *n*	21.57±9.90	19.56±11.19	20.41±9.93	18.28±8.41	22.15±9.61	0.136
MII, *n*	18.23±8.96	17.13±10.53	17.11±8.47	15.80±7.35	18.67±8.84	0.334
2PN, *n*	11.54±7.32	11.49±7.13	11.51±6.53	9.86±5.11	12.39±7.06	0.278
Embryo, *n*	10.49±6.55	10.00±6.09	10.29±5.87	8.64±4.03	11.04±6.53	0.211
Fertilisation rate	704/1102 (63.13)	517/771 (67.05)	725/1078 (66.69)	493/790 (62.40)	1648/2483 66.37	0.864
Implantation rate	17/120 (18.50%)	14/88 (15.90%)	12/129 (9.30%)	17/108 (15.74%)	49/271 (18.08%)	0.605
Clinical pregnancy	15 (37.4%)	10 (22.2%)	11 (17.5%)	13 (26.0%)	41 (30.8%)	0.356
Abortion rate	4 (8.45%)	2 (4.4%)	1 (1.6%)	0 (0.0%)	6 (4.5%)	0.353

Data extracted by one-way ANOVA test. $P \leq 0.05$ was considered statistically significant. Note: MII=number of matured oocytes, 2PN=number of fertilised oocytes, two pronuclei, fertilisation rate: 2PN number per the number, MII=implantation rate: gestational sac in sonography per the number of embryos transferred embryos

outcome. Lu, in 2010, found that decreased basal testosterone levels had negative effects on implantation and reduced embryo implantation in women with weak ovarian reserve.[15] The results of the present study showed that androgen levels were not an effective predictor for pregnancy outcomes in PCOS women. Though the relationship between basal testosterone levels and reservation of ovaries is confirmed, perhaps the rate of pregnancy can change by the levels of testosterone in PCOS women. Also, in patients with high levels of testosterone, the rate of pregnancy loss did not increase. However, maybe free androgen index (FAI) elevation was a predictive parameter for later miscarriage in recurrent pregnancy loss cases.[16]

CONCLUSION

In conclusion, in our present study, serum testosterone levels did not show any correlation with pregnancy outcomes in ART cycles of PCOS patients. However, basal testosterone levels is a good predictor for ovarian reserve and ovarian response. Consequently, we suggest a prospective study is needed to approve the role of testosterone in the prediction of pregnancy outcomes in ART cycles.

Conflicts of interest

There are no conflicts of interest.

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