

Basal serum testosterone levels correlate with ovarian response but do not predict pregnancy outcome in non-PCOS women undergoing IVF

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Abstract

Purpose To evaluate basal testosterone (T) levels in women undergoing in vitro fertilization (IVF) cycles and examine the association between basal T levels and ovarian response or IVF pregnancy outcome.

Methods We retrospectively analyzed 1413 infertile Chinese women undergoing their first IVF treatment at our institution's reproductive center from March 2011 to May 2013. The basal testosterone (T) levels in women undergoing in vitro fertilization (IVF) and the relationship between basal T levels and ovarian response or IVF pregnancy outcome were determined. These patients did not have polycystic ovary syndrome (PCOS) or endometriosis, and were treated with a long luteal down-regulation protocol. Subjects were divided into 2 groups according to basal testosterone (T) levels: Group 1, basal T values <20 ng/dl ($n=473$), and Group 2, basal T values >20 ng/dl ($n=940$). We evaluated the association of basal T levels with ovarian response and IVF outcome in the two groups.

Results In this study, BMI, basal follicle-stimulating hormone (FSH) levels, basal luteinizing hormone (LH) levels, antral follicle count (AFC), days of stimulation, total gonadotrophin

dose, basal FSH/LH ratio, and the number of follicles >14 mm were significantly different ($P<0.05$) between the two groups. Basal T level positively correlated with ovarian reserve function, number of follicles >14 mm on human chorionic gonadotrophin (HCG) day, and total gonadotropin dose. However, basal T levels play no role in predicting IVF pregnancy outcome.

Conclusion Basal T level can be used as a good predictor for ovarian response and the number of large follicles on HCG day. Additionally, we may use basal T level as a marker to predict FSH dosage. In general women, lower level of T might relate with potential poor ovarian response. However, based on our data, basal T levels do not predict pregnancy outcome.

Keywords IVF · Pregnancy outcomes · Basal testosterone (T) levels · Ovarian response

Introduction

Assisted reproductive technologies (ARTs) are increasingly being used in infertility treatments. Because success with controlled ovarian hyperstimulation (COH) depends on the ability to recruit adequate numbers of follicles, ovarian response is a key factor in in vitro fertilization (IVF) cycles. Although ovarian response can be predicted by many markers including age, antral follicle count (AFC), basal serum follicle-stimulating hormone (FSH), serum inhibin B, and serum anti-Müllerian hormone (AMH), many patients who have normal parameters have poor gonadotropin responsiveness and low-quality oocytes and embryos [1–3]. Therefore, it becomes a challenge to predict ovarian response before undergoing expensive IVF treatments.

Testosterone and dehydroepiandrosterone sulfate (DHEAS) are two important hormones in few studies that

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specify the effect of androgens on IVF cycles [4–6]. In some studies, exogenous testosterone used for patients who had poor ovarian response improved IVF treatment success [7]. We hypothesized whether serum testosterone levels could predict ovarian response and IVF outcome. To the best of our knowledge, the application of basal serum T levels for predicting ovarian response and pregnancy outcome is debated. Yingying Qin's group suggested that basal T level was a predictor for ovarian response and pregnancy outcome in women with diminished ovarian reserve; but could not in those with normal serum FSH [8]. However, Fratharelli and Peterson's study showed that patients with Day 3 testosterone level lesser than 20 ng/dl were five time less likely to achieve pregnancy [9]. This might be explained by limited sample size and heterogeneous population mixed by patients with polycystic ovary syndrome (PCOS) and/or endometriosis as PCOS and endometriosis are associated with higher or lower serum T levels.

However, few studies have evaluated non-hyperandrogenic women and presumed ovarian reserve in general populations. Thus, conclusive and definite data regarding the association between basal serum T levels with ovarian response and IVF outcome are still lacking. With this background, we sought to investigate whether serum T levels could predict ovarian response and IVF outcome in non-PCOS women.

Materials and methods

Study population

The relationship between basal T levels and ovarian response or IVF outcome was determined. The ethics committee of First Affiliated Hospital of Zhengzhou University approved this retrospective study. At the same time, the participants provided written consent for the data to be stored and used for research. Chinese patients ($n=1413$) who underwent their first IVF-embryo transfer (ET) cycle at the Center for Reproductive Medicine, The First Affiliated Hospital, Zhengzhou University from March 2011 to May 2013 were included in the study. We only selected women who used the long protocol to avoid possible bias from different protocols. Patients with endometriosis and PCOS were excluded from this study. Patients that were older than 42 years of age or with FSH levels >12 IU/L were also excluded. The age of patients varied from 21 to 42 years. According to Fratharelli and Peterson's work [9], patients with testosterone levels less than 20 ng/dl on day 3 were five times less likely to achieve success in IVF treatment. Patients were divided into 2 groups. Group 1 included patients who had basal T values <20 ng/dl ($n=473$) and Group 2 had basal T values >20 ng/dl ($n=940$).

Study protocol

The long luteal down-regulation protocol was used in all patients. Briefly, the gonadotropin-releasing hormone (GnRH) agonist (0.1 mg/ampoule Tryptorelin, Ferring, Germany) was administered in the mid-luteal phase of the previous cycle until the day of HCG administration. After using GnRH for 14 days and when satisfactory pituitary desensitization was achieved (serum E2 levels lower than 50 pg/ml, FSH <5 IU/L, LH <5 IU/L, size of follicular <10 mm, and endometrial thickness <5 mm), gonadotropin stimulation was started with daily use of recombinant FSH (75 U/ampoule, Gonal F, Serono. Ltd, Switzerland). The starting gonadotrophin dose was according to age, AFC, and basal hormone levels (20–25y:112.5–150 U/ampoule; 25–30y:150–225.5 U/ampoule; 30–35y:225.5–300U/ampoule; >35 y:300–400U/ampoule, FSH levels >12 IU/L were excluded). It was adjusted according to follicular development observed by transvaginal ultrasonography and serum E2 concentrations. When one follicle reached 16 mm in diameter, human menopausal gonadotrophins (HMG) (75 U/ampoule, Ferring GmbH, Germany) was administered. HCG (2,000 IU) (Lizhu. Ltd, Guangdong, China) and recombinant HCG (250 μ g) (Serono. Ltd, Switzerland) was administered when at least two follicles reached 18 mm or one follicle reached 20 mm in diameter. Oocytes were retrieved by transvaginal ultrasound-guided follicular aspiration after 36–37 h. Up to three embryos per patient were transferred (2 embryos were transferred for women aged <35 years old and in their first treatment cycle, 3 embryos were transferred for women aged ≥ 35 years old or with ≥ 2 treatment cycles) 2 or 3 days after oocyte retrieval and the luteal phase was supported with 60 mg progesterone (20 mg/ampoule, Xianju Ltd., Zhejiang, China). A positive pregnancy test was defined by >5 IU/L of plasma β -hCG 14 days after embryo transfer. Spontaneous miscarriage was defined both biochemically and clinically. Good quality embryos were defined as embryos that developed from normal fertilized eggs with no fragmentation or no more than 1/3 fragmentation, the absence of multinucleation, 3 to 5 blastomeres 48 h after egg retrieval, and at least 7 blastomeres 72 h after egg retrieval [10]. Patients did not use androgen supplementation.

Hormone assays

Basal serum levels of T, FSH, E2, LH, and prolactin (PRL) were measured on day 2 or 3 of the menstrual cycle from each subject. β -hCG levels were measured 14 days after embryo transfer. All basal hormone assays were performed using the electrochemiluminescent immunoassay in the analyzer cobas e 601 (Roche, Switzerland). The analytical sensitivities of basal T, FSH, LH, E2, and β -hCG levels were 0.42 ng/ml, <0.10 mIU/ml, 0.10mIU/ml, 5.0 pg/ml, and <0.1 mIU/ml, respectively.

Statistical analyses

Student’s *t*-test and analysis of covariance (ANCOVA) were performed to compare the means between two groups. Pearson correlation tests and Spearman’s correlation analyses were used to assess the relationship between basal T levels and continuous parameters. Multiple linear regression analyses were used to find possible independent determinants of test variables among parameters showing significant correlations and their contributions. The area under the receiver operating characteristic curve (ROC) was examined for the predictive value of pregnancy outcome. Data were analyzed using the SPSS statistical package (SPSS version 13.0, Chicago, IL). All data are reported as the mean with their associated standard deviations and all tests were two-tailed. *P*<0.05 was considered statistically significant.

Results

Table 1 shows the comparison of characteristics and COH parameters between the two groups. BMI, basal FSH levels, basal LH levels, AFC, days of stimulation, total gonadotrophin dose, basal FSH/LH ratio, and the number of follicles >14 mm were significantly different (*P*<0.05). The patients in Group 2 had more oocytes retrieved, good quality embryos, and embryos cryopreserved. However, the pregnancy

outcomes were not significantly different between the two groups. There were positive associations between basal T levels and age, BMI, basal FSH levels, basal LH levels, AFC, starting dose of gonadotropins, total dose of gonadotropins, the number of follicles >14 mm, number of oocytes retrieved, number of good quality embryos, and number of embryos cryopreserved (Table 2).

In rank correlation analyses, the characteristics associated with AFC were age, basal T levels, basal FSH levels; basal LH levels (data not shown). The characteristics associated with the number of follicles >14 mm on HCG day were age, BMI, AFC, basal T levels, basal FSH levels, basal LH levels, total gonadotropin dose, and days of stimulation (data not shown). The characteristics correlated with total dose of gonadotropins were age, BMI, basal T levels, basal FSH levels, basal LH levels, and AFC (data not shown).

Accordingly, multiple linear regression analyses were performed to study the major independent factors for AFC, number of follicles >14 mm on HCG day, and total dose of gonadotropins which were used as the dependent variables, whereas the independent variables included the characteristics showing significant correlations mentioned above, respectively (Tables 3, 4, and 5). Basal T level was an independent factor for AFC, number of follicles >14 mm on HCG day, and total dose of gonadotropins. For the number of good quality embryos and number of embryos cryopreserved, the only independent factor was number of oocytes retrieved (data not

Table 1 Comparison of characteristics and COH parameters between group 1 and group 2 patients

Variables	Group 1 (<i>T</i> <20 ng/dl, <i>n</i> =473)	Group 2 (<i>T</i> >20 ng/dl, <i>n</i> =940)	<i>P</i> value
Age (years)	30.94±4.42	30.88±4.37	0.805
BMI (kg/m ²)	21.94±2.87	22.51±3.13	0.001
Pregnancy history	4.38±3.25	4.41±3.20	0.854
Basal FSH levels (IU/L)	7.63±1.64	7.18±1.57	0.000
Basal LH levels (IU/L)	5.04±2.20	5.62±3.31	0.001
Basal E2 levels (pg/ml)	42.40±41.39	42.01±37.93	0.860
Basal PRL levels	17.92±25.64	20.86±33.32	0.092
Antral follicle count	4.99±2.57	5.68±2.87	0.000
Basal FSH/LH ratio	1.89±3.07	1.56±0.744	0.002
Starting dose of gonadotropins (IU)	178.65±50.05	174.23±49.99	0.117
Days of stimulation	11.20±1.74	10.93±1.61	0.004
Total gonadotropin dose (IU)	2057.21±755.02	1904.70±682.00	0.000
Number of follicles >14 mm	9.11±3.10	10.67±3.41	0.000
Number of oocytes retrieved	10.67±4.69	12.51±5.28	0.000
Endometrial thickness on HCG day (cm)	12.55±2.30	12.26±2.29	0.027
Number of embryos transferred	1.93±0.40	1.93±0.46	0.981
Number of good quality embryos	5.48±3.27	6.63±3.79	0.000
Number of embryos cryopreserved	3.69±3.21	4.83±3.79	0.000
Pregnancy outcome rates	53.27 % (252/473)	55.63 % (523/940)	0.400

P<0.05

Table 2 Spearman correlation analyses of associations between basal T level and patient characteristics and COH parameters

Variables	r value	P value
Age (years)	-0.073	0.006
BMI (kg/m ²)	0.109	0.000
Basal LH levels (IU/L)	0.121	0.000
Basal FSH levels (IU/L)	-0.180	0.000
Basal E2 levels (pg/ml)	0.029	0.277
Basal PRL levels (pg/ml)	0.045	0.088
Basal FSH/LH ratio	-0.201	0.000
Antral follicle count	0.184	0.000
Starting gonadotropin dose (IU)	-0.122	0.000
Number of oocytes retrieved	0.280	0.000
Number of follicles >14 mm	0.370	0.000
Days of stimulation	-0.039	0.143
Total dose of gonadotropins (IU)	-0.122	0.000
Endometrial thickness on HCG day (cm)	-0.039	0.144
Number of good quality embryos	0.223	0.000
Number of embryos cryopreserved	0.220	0.000
Live births	-0.021	0.426

$P < 0.01$

shown). For AFC, the influencing independent factor in order of decreasing importance were age, basal FSH levels, basal T levels, and basal LH levels. For the number of follicles >14 mm on HCG day, the influencing independent factor in order of decreasing importance were basal T levels, total dose of gonadotropins, basal LH levels, AFC, and basal FSH levels. For total gonadotropin dose, it was age, basal FSH levels, BMI, AFC, and basal T levels in order of decreasing importance.

ROC curve analyses of the diagnostic accuracy of discrimination between pregnancy per transfer versus non-pregnancy per transfer were also performed. In the ROC model, the area under the receiver operating characteristic curve was 0.512. Basal T levels play no role in predicting IVF pregnancy outcome (data not shown).

Discussion

Androgens, serving as precursors for ovarian estrogen synthesis, also have a fundamental trophic role in primate ovarian follicular development by augmenting follicular stimulating hormone (FSH) receptor expression on granulosa cells [11]. Androgen receptors (AR) are rich in the granulosa cells of healthy prenatal and antral follicles. It appears that follicular androgen levels significantly affect granulosa cell function [12]. In vitro data indicate that human granulosa cells possess sulfates activity, and that DHEAS can be used as a substrate for both estrogen and androstenedione synthesis [13, 14]. Androgens can augment FSH receptor expression in granulosa cells and are thought to promote follicular growth and estrogen biosynthesis by amplifying the effects of FSH in rhesus monkeys [15]. Studies on murine models of conditional granulosa cell and oocyte-specific deletion of the androgen receptor found positive correlations between androgen receptor and FSH receptor expression, suggesting that androgens may prevent preantral follicle growth and atresia [16–20]. Similarly, it has recently been shown that androgen receptor mRNA and androgen levels in follicular fluid correlate with FSH receptor mRNA expression in human granulosa cells from small antral follicles [21]. Androgens also increase insulin-like growth factor 1 (IGF-1) expression in the primate ovary, which plays an essential role in regulating follicular development [22–24]. In mechanism, basal serum testosterone may have the patient for predicting ovarian response.

In our study, the data suggests that BMI, FSH, LH, AFC, days of stimulation, total gonadotrophin dose, basal FSH/LH ratio, and the number of follicles >14 mm were significantly different between the groups. Basal T levels positively correlated with ovarian reserve function, the number of follicles >14 mm on human chorionic gonadotrophin (HCG) day, and total gonadotropin dose. The basal T level is a good predictor for ovarian reserve function, along with the number of follicles >14 mm on HCG day and total gonadotropin dose. Basal T levels are helpful for determining the dosage of gonadotropins, and subsequently allow an individualized COH strategy before entering IVF cycles. This is consistent with data from

Table 3 Multiple linear regression analyses of possible determinants for AFC

The dependent variable is AFC. The model ($r=0.343$, $R^2=0.118$, adjusted $R^2=0.115$, $P<0.001$) The values of the standardized coefficients reflect the independent contributions of each predictor to dependent variables

Independent variables	Coefficients				
	Unstandardized coefficients		Standardized coefficients		
	β	Std. Error	β	t	P value
(constant)	9.589	0.622		15.409	0.000
Age (years)	-0.126	0.016	-0.198	-7.798	0.000
Basal FSH levels (IU/L)	-0.235	0.045	-0.136	-5.182	0.000
Basal LH levels (IU/L)	0.077	0.024	0.083	3.168	0.002
Basal T levels (ng/dl)	0.043	0.007	0.160	6.039	0.000

Table 4 Multiple linear regression analyses of possible determinants for the number of follicles >14 mm

The dependent variable is number of follicles >14 mm. The model ($r=0.573$, $R^2=0.328$, adjusted $R^2=0.326$, $P<0.001$)
The values of the standardized coefficients reflect the independent contribution of each predictor to dependent variables

Independent Variables (constant)	Unstandardized coefficients		Standardized coefficients		
	β	Std. Error	β	t	P value
(constant)	5.933	0.629		9.438	0.000
AFC	0.139	0.029	0.115	4.816	0.000
Basal FSH levels (IU/L)	-0.210	0.050	-0.100	-4.208	0.000
Basal LH levels (IU/L)	0.150	0.026	0.132	5.756	0.000
Basal T levels (ng/dl)	0.123	0.008	0.376	16.013	0.000
Total gonadotropin dose	-0.001	0.000	-0.228	-7.563	0.000
Days of stimulation	0.296	0.058	0.145	5.092	0.000

the studies mentioned above. However, based on our data, basal T levels do not predict pregnancy outcome.

To our knowledge, the good functional ovarian reserve is the basis of follicular response and success rates. The prediction of ovarian reserve is equally important with the ovarian response. Serum and ultrasonographic markers have been tested to infer the gonadal reserve of infertile women, but none has been proven to reflect the complex follicular dynamics or be strongly correlated with the size and/or quality of the primordial follicles remaining in the gonads. Thus, these tests do not ideally reflect the pool of unrecruited follicles, which may be responsible for ovulatory cycles and the long-term reproductive potential [25–28]. Age, menstrual cycle length, basal serum FSH, basal estradiol, clomiphene citrate challenge test, AFC, ovarian volume, and ovarian blood flow had been reported to evaluate ovarian reserve, but we need more markers to make the cycle cheaper and more likely to be successful [29–34]. In this study, we find that basal T level correlates with AFC and basal FSH levels. Our data suggest that we can use basal T levels as a new marker to predict ovarian reserve, together with the parameters above, but it does not have obvious advantages over FSH-AMH. However, more studies are needed to confirm these results.

To gain further insight into the role of basal T levels, it can be as one marker to predict days of FSH and its dosages. In vitro fertilization (IVF) is the last resort treatment for many

subfertile couples and it is very expensive; therefore, it is important for reproductive physicians to find reasons for poor responses and help couples select the best individualized strategy for controlled ovarian hyperstimulation (COH). COH is an essential part of IVF and is needed to obtain oocytes that can be fertilized in vitro. The high cost of IVF is partly due to the use of expensive drugs, such as gonadotrophins, needed for COH [34, 35]. In clinical practice, physicians often rely on clinical experience and personal judgment when selecting an appropriate starting dose of follicle stimulation hormone (FSH). Defining the optimal dose of FSH is complicated. However, women with hyper-responses to ovarian stimulation are at risk of increased patient discomfort, cycle cancellation, and developing ovarian hyperstimulation syndrome (OHSS). Various ovarian reserve tests (ORTs) are available, and basal FSH is the oldest. The antral follicle count (AFC) and serum levels of anti-Müllerian hormone (AMH) are able to predict ovarian response to COH, allowing for studies evaluating the value of adjusting FSH dosage based on these parameters [36–41]. In this study, no significant association was found between basal T levels and oocyte retrieved, but basal T level is an independent factor for both days of stimulation and total dose of gonadotropins. That is, in order to reach similar ovarian responses, patients with lower basal T levels needed more days and more doses of gonadotropins.

Table 5 Multiple linear regression analyses of possible determinants for total gonadotropin dose

The dependent variable is the total dose of gonadotropins. The model ($r=0.662$, $R^2=0.438$, adjusted $R^2=0.436$, $P<0.001$)
The values of the standardized coefficients reflect the independent contribution of each predictor to dependent variables

Independent variables (constant)	Unstandardized coefficients		Standardized coefficients		
	β	Std. Error	β	t	P value
(constant)	-1671.608	163.101		-10.249	0.000
Age (years)	-83.263	3.405	0.514	24.450	0.000
BMI	32.494	4.769	0.140	6.814	0.000
AFC	-31.485	5.388	-0.124	-5.893	0.000
Basal FSH levels (IU/L)	85.386	9.179	0.194	9.302	0.002
Basal T levels (ng/dl)	-5.081	1.434	-0.074	-3.542	0.000

However, the use of basal serum T levels for predicting pregnancy outcome is debated. Most studies have suggested that basal serum T levels have no relationship with IVF outcome [42, 43]. However, a few have suggested that basal serum T levels could predict pregnancy outcome in the patients with diminished ovarian reserve. Rashidi's group found that T levels were significantly elevated in pregnant women on the 14th day of embryo transfer, suggesting serum testosterone, but not basal serum T levels, may play a role in predicting pregnancy outcome. The different conclusion among the studies might be due to the small sample sizes. Therefore, we should investigate further the effect of the serum testosterone. The data from our study showed that women who had lower basal serum T levels had more oocytes retrieved, good quality embryos, and embryos cryopreserved. However, we also did not find a significant basal serum testosterone level at which pregnancy outcome was affected.

A weakness of this study is only basal serum T level, which is an ovarian androgen, was evaluated. DHEA and other adrenal androgens are also very important [43]. A previous study suggested that age, BMI, and causes of infertility may involve ovarian response and/or pregnancy outcome [44]. We adjusted for confounding variables including age, treatment cycles, infertility history, and starting gonadotropin dose, but not BMI, considering their possible associations with basal serum T levels. Another study suggested that BMI was related with basal serum T levels [10]. The effect of BMI needs further explanation.

Most studies of serum T levels in infertile women have focused on women with irregular cycles and PCOS. In this study, women with irregular menstrual cycles or a known diagnosis of PCOS were excluded. Another strength of our study is the large sample size ($n=1413$). The results from the study indicate that it is important to evaluate basal serum testosterone levels.

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