Basal Testosterone and Sex Hormone Binding Globulin Levels in the Prediction of Stimulation Parameters and Cycle Outcome in Cycling Patients Undergoing in Vitro Fertilization

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OBJECTIVE: Basal serum testosterone (T) and sex hormone binding globulin (SHBG) levels in regularly cycling patients who undergone in vitro fertilization (IVF) treatment and their relations with stimulation parameters and IVF outcomes.

STUDY DESING: Two hundred patients seeking their first IVF treatment from June 2007 to January 2008 were evaluated prospectively. Patients aged 23-39 with regular menstrual cycles and with FSH levels less than 12 mIU/ml were included. Basal concentrations of FSH, LH, E2, PRL, TSH with T and SHBG were determined. Free androgen index (FAI) was calculated. IVF stimulation parameters, fertilization and clinical pregnancy rates were evaluated.

RESULTS: History of smoking was significantly associated with an increase in T. T had significantly positive association with the number of oocytes retrieved and the number of embryos transferred. However, neither T nor SHBG predicted fertilization or clinical pregnancy rates.

CONCLUSION: Basal serum T levels during IVF have a positive correlation with part of the stimulation parameters including number of oocytes retrieved and the number of oocytes transferred. However, neither T nor SHBG has any affect on fertilization or pregnancy rates.

Key Words: Testosterone, SHBG, Basal hormones, IVF parameters, Pregnancy outcome

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Introduction

Many indicators have been proposed to help in predicting the success of an in vitro fertilization (IVF) cycle. The majority of the studies in the literature have evaluated the age, body mass index (BMI), smoking status, basal endocrine profile and ultrasonographic markers such as mean ovarian volume and antral follicule count as well as provocative challenge tests as predictors of IVF outcome. The contribution of these tests to prediction of ovarian response or to success in assisted reproduction has been demonstrated by a number of investigators. Despite their increasing use in clinical practice, none has been shown to be an accurate marker. The main shortcoming about the hormonal tests has been the limited predictive value of a normal result with many patients who have poor gonadotropin responsiveness and low-quality oocytes and embryos having normal screening results.[1]

It is postulated that androgen levels can predict stimulation parameters during an IVF cycle and pregnancy outcomes. Frattarelli et al demonstrated that basal testosterone (T) levels were associated with many stimulation parameters including day 3 E2, number of ampules used, and duration of stimulation. Day 3 T levels greater than 20 ng/dL were associated with successful pregnancy rate during an IVF cycle.[2]

T is the most important circulating and naturally occuring androgen in both men and women. In women, T is produced primarily through peripheral conversion of androstenedione (50%) with the remainder of production concentrated in the ovary (25%) and adrenal cortex (25%).[3] T circulates in 3 forms which is free, bound to albumin and bound to sex hormone binding globulin (SHBG). The ratio between T and SHBG concentrations in serum (free androgen index, FAI) is used as an index of biologically active T. This ratio correlates well to values for free or non-SHBG-bound T determined by physicochemical methods.[4]

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We measured basal serum T and SHBG levels in regularly cycling patients who undergone IVF treatment and evaluated their relations with stimulation parameters and IVF outcomes.

Material and Method

Two hundred patients seeking their first IVF treatment from June 2007 to January 2008 were evaluated prospectively. Patients aged 23-39 with regular menstrual cycles and with FSH levels less than 12 mIU/ml were included in the study. Regular menstrual cycles were defined as 24-35 days in length. Patients with known endocrinopathies (e.g., polycystic ovarian disease, amenorrhea, hyperprolactinemia, thyroid disease) were excluded from the study. The study was approved by the instutional review board. All basal serum specimens from the patients prior to initiating an IVF cycle were obtained during days 3 to 5 of the menstrual cycle under standardized conditions in the morning between 8 and 10 a.m. in the fasting state. Basal concentrations of FSH, LH, E2, PRL and TSH were determined. Hormonal analysis were performed using commercially available radioimmunoassays. Part of the blood samples were centrifuged immediately after sample collection and stored at -80 ºC until assayed for T and SHBG. SHBG was measured using a fully automated system (Immulyte 2000; Siemens). The method is a solid-phase two-site chemiluminescent enzyme immunometric assay. The analytic sensitivity was 0.02 nmol/L. Free androgen index (FAI) was calculated by the formula of [T (nmol/L) X 100]/SHBG(nmol/L).[5]

Antral follicle count, duration of ovulation induction, E2 level and follicle count on the day of HCG injection, number of oocytes retrieved, number of oocytes with two pronuclei, number of embryos transferred, fertilization and clinical pregnancy rates were noted for each patient. The fertilization rate was defined as the number of oocytes with two pronuclei after insemination divided by the number of oocytes inseminated. Clinical pregnancy was defined as a positive serum BHCG result with ultrasound evidence of a gestational sac and fetal heart.

Statistical Analysis

Data were evaluated with using SPSS for Windows release 11.5 package programme (Chicago Inc. USA). T test for independent samples was used to compare nominal variables with two categories. Pearson and Spearman rank correlation analysis were used to determine relationships between variables.

Alpha values has accepted 0.05 for statistically significant. As descriptive statistics, mean±SD values were given for continuous data, number and percentages were given for nominal data.

Results

Demographic data and IVF parameters of the patients enrolled in the study are given in Table 1. The mean age of the patients were 29.5±4.5 years (range 23 to 39 years). The mean duration of infertility was 8.4±4.3 years. Indications for IVF were male factor in 108 (54%) patients, unexplained infertility in 80 (40%) patients, tubal factor in 8 (4%) patients and endometriosis in 4 (2%) patients. A mean of 9.1±4.7 oocytes per patient were recovered. The fertilization rate was 63.5%. A mean of 2.6±1.2 embryos were transferred into the uterus. Clinical pregnancy rate was 42%.

Table 1: In vitro fertilization parameters

Parameter	
Age (years, mean±SD)	29.5±4.5
Duration of infertility (years, mean±SD)	8.4±4.3
E2 (pg/ml, mean±SD)	41.4±15.6
FSH (mIU/ml, mean±SD)	7.3±2.2
LH (uIU/ml, mean±SD)	5.2±2.7
TSH (uIU/ml, mean±SD)	3.4±5.5
T (ng/dL, mean±SD)	42±0.23
SHBG (nmol/L, mean±SD)	42.9±27.04
FAI (mean±SD)	1.2±0.9
Antral follicle count (n, mean±SD)	9.1±3.01
Duration of ovulation induction (days, mean±SD)	10.5±1.9
Number of oocytes retrieved (n, mean±SD)	9.1±4.7
Number of oocytes with two pronuclei (n, mean±SD)	3.8±2.8
Number of embryos transferred (n, mean±SD)	2.6±1.2
Fertilization rate (%±SD)	63.5%±24.1%
Cycle cancellation (n, %)	12 (6%)
Fertilization failure (n,%)	8 (4%)
Clinical pregnancy rate (n, %)	84 (42%)

The overall mean serum T and SHBG was 42±23 ng/dL and 42.9±27.04 nmol/L respectively. Table 2 shows the correlation coefficients between T, SHBG, and FAI and stimulation parameters during the IVF cycle including E2 level on day of HCG injection, number of oocytes retrieved, number of oocytes with two pronuclei, number of embryos transferred and fertilization rate. Advancing age was associated with a decrease in T, SHBG and FAI, but it was not statistically significant. T was positively correlated with E2 level on the day of HCG injection, number of oocytes with two pronuclei and fertilization rate but they were not statistically significant either. The only significant positive correlation was found between T and the number of oocytes retrieved and the number of embryos transferred. SHBG was found to be positively correlated with E2 level on the day of HCG injection, number of oocytes retrieved, and fertilization rate and negatively correlated with number of oocytes with two pronuclei and the number of embryos transferred, but the results were not statistically significant. FAI was positively correlated with E2 level on the day of HCG injection, number of oocytes retrieved, number of oocytes with two pronuclei, fertilization rate and the number of embryos transferred, but again the associations were not statistically significant.

History of smoking was significantly associated with an increase in T. There was a borderline significant association between fertilization failure and SHBG, being lower in patients with fertilization failure. Among the patients with cycles cancelled, T and FAI was found to be lower. Cycle cancellation had a borderline significant association with T and a significant association with FAI. However, neither T nor SHBG were significantly correlated with clinical pregnancy rates (Table 3).

Table 2: Correlations between hormones and stimulation parameters

	Testosterone	SHBG	FAI
Testosterone			
r		-0.085	0.774
p		0.398	**0,001**
SHBG			
r			-0.650
p			**0,001**
E_2			
r	0.034	0.081	0.018
p	0.736	0.420	0.859
FSH			
r	-0.09	-0.027	-0.041
p	0.375	0.793	0.688
LH			
r	0.161	0.066	0.110
p	0.110	0.517	0.275
Antral follicle count			
r	0.184	0.036	0.149
p	0.067	0.725	0.139
E_2 level on day of HCG			
r	0.152	0.048	0.114
p	0.140	0.647	0.269
Number of oocytes retrieved			
r	0.238	0.070	0.169
p	**0.022***	0.506	0.107
Number of oocytes with two pronuclei			
r	0.176	-0.060	0.180
p	0.106	0.582	0.097
Number of embryos transferred			
r	0.221	-0.016	0.166
p	**0.040***	0.881	0.129
Fertilization rate			
r	0.053	0.025	0.065
p	0.618	0.813	0.541

**p<0.05, significant.*

Table 3. T, SHBG levels and FAI in cycle and pregnancy outcomes

	Testosterone	SHBG	FAI
Cycle cancellation			
Yes (n=12)	29±11	53.2±34.6	0.76±0.64
No (n=188)	43±24	41.5±25.7	1.3±0.92
P	0.059	0.112	**0.013***
Fertilization failure			
Yes (n=8)	44±14	29.3±11.4	1.7±0.82
No (n=192)	40±24	44.1±27.7	1.2±0.9
P	0.364	0.053	0.094
Clinical pregnancy			
Yes (n=84)	44±26	40.8±21.5	1.3±0.82
No (n=116)	41±23	43.2±27.8	1.2±0.92
P	0.766	0.937	0.718
Smoking			
Yes (n=22)	53±22	37.4±20.9	1.7±0.94
No (n=178)	40±23	43.6±27.7	1.2±0.89
P	**0.043***	0.418	0.055

**p<0.05, significant*

Discussion

It is postulated that androgen levels can predict stimulation parameters during an IVF cycle and pregnancy outcomes. There are several studies in the literature which evaluated the androgen levels to determine their predictive value to the cycle IVF parameters and/or cycle outcome. Most of them have focused on women with irregular cycles and PCOS. The others have demonstrated different results about the association between T, SHBG and cycle outcomes. In this study women with irregular cycles and with known endocrinopathies were excluded.

In the present study, advancing age was associated with a decrease in serum T but it was not statistically significant. One of the first studies to examine the correlates of T concentrations in pre-and perimenopausal women (i.e., age, menopausal status, body composition, and lifestyle behaviors) was the Michigan Bone Health Study (1992-1995) which was conducted among 611 women aged 25-50 years. In that study the authors did not find a significant change in T concentrations with advancing age.[6] Burger et al in a study of 172 women from 45 to 55 years of age, followed for 7 years through a natural menopause, did not observe a decrease in serum T, but did observe a decrease in serum SHBG.[7] However, in the study of Barbieri et al it was found that serum T levels decreased with advancing age.[8] They explained this result by the decrease of LH stimulation of ovarian androgens by the decade of the 30s.

Consistent with our findings, several other studies have demonstrated that smoking was associated with increased serum T levels. In the Michigan Bone Health Study smoking behavior was associated with increased serum T levels. Current smokers had the highest mean levels, with mean total T concentrations decreasing in former and nonsmokers.[6] However, Thomas et al compared estradiol, as well as androgen and salivary progesterone concentrations in 25 normal premenopausal smokers and 21 nonsmokers measured in a single menstrual cycle. They found no significant differences in plasma T, androstenedione, and dehydroepiandrostenedione concentrations.[9] On the other hand, Barbieri et al found that years of cigarette smoking were associated with increased serum T levels in women undergoing IVF.[8] The acute hormonal response to smoking is similar to that in acute stres, with activation of the pituitary and sympatho-adrenal system.[4] It is demonstrated that greater concentrations of T observed in smokers were due to low metabolic clearance rates for androstenedione, T, estrone, and estradiol.[10] Increased circulating carbon monoxide concentrations in smokers inhibit P-450 hydroxylases which are responsible for metabolism of steroid hormones.[6]

Androgen levels at basal serum of patients undergoing IVF have been evaluated in several studies. There is inconsistency among the results of the studies in the literature. Frattarelli et al demonstrated that patients with day 3 levels ≤20 ng/dL were five times less likely to achieve pregnancy.[2] In a latter study of Frattarelli et al it was found that serum androgen levels during IVF correlate with IVF stimulation parameters, but they did not predict pregnancy rates. In that study basal T levels significantly correlated with multiple stimulation paramters such as ovarian volume, basal antral follicles, BMI, follicles, peak E2 level, oocyte number, mature oocytes, and amount of go-

nadotropins used .[1] One of the recent studies conducted with a large number of cycling IVF patients by Barbieri et al demonstrated that serum T correlated positively with pre-HCG serum E2 and number of oocytes retrieved. However, serum T did not significantly influence fertilization or pregnancy rates.[8] Zollner et al found that patients who conceived had a significantly lower T compared to patients who did not.[11] In our study of regularly cycling women, we found significant positive correlation only between serum T and both the number of oocytes retrieved and number of embryos transferred, but there was no relationship between serum T and fertilization rate nor pregnancy rate. Our results add to the current literature, but further studies with larger population are needed to clarify the role of basal serum T on stimulation parameters and pregnancy outcome.

SHBG, primarily produced by the liver, is the major determinant of the biologically available E2 and T in human plasma. The plasma concentration of SHBG reflects the balance between estrogen and androgen. It has been suggested that SHBG may modulate the bioavailability of ovarian steroids to affect pregnancy outcome during IVF treatment. Lin et al found that increases in plasma SHBG levels occur throughout the follicular and luteal phases of COH cycles for assisted reproduction.[12] They suggested that the increase in SHBG was due to the influence of an increasing secretion of supraphysiological E2 on hepatic synthesis. The mean SHBG levels and E2/T ratios were significantly higher and the T levels and FAI values were significantly lower in the luteal phase of those with a successful pregnancy as compared to those who did not conceive after IVF. SHBG influences the relative circulating estrogen and androgen balance, thus pregnancy outcome. However, in that study no significant difference was found in early follicular level of SHBG between pregnant and non pregnant women. In the present study, we found no significant correlation between basal serum SHBG and any of the stimulation parameters nor fertilization and pregnancy rates.

In conclusion, basal serum T levels during IVF have a positive correlation with part of the stimulation parameters including number of oocytes retrieved and the number of oocytes transferred. However, neither T nor SHBG predict fertilization or clinical pregnancy rates. Our study suggests no value to the routine measurement of T or SHBG in IVF patients in regularly cycling women without PCOS.

İn Vitro Fertilizasyon Tedavisi Alan Regüler Menstrüel Siklusu Olan Hastalarda Bazal Testosteron ve Seks Hormon Bağlayıcı Globulin Seviyelerinin Stimülasyon Parametrelerini ve Siklus Sonuçlarını Öngörmedeki Yeri

AMAÇ: İn vitro fertilizasyon (IVF) tedavisi alan regüler menstrüel siklusu olan hastalarda bazal testosteron (T) ve seks hormon bağlayıcı globulin seviyeleri (SHBG) ile stimülasyon parametreleri ve IVF sonuçları ile ilişkisi.

GEREÇ VE YÖNTEM: Haziran 2007 ve Ocak 2008 arasında ilk siklus IVF tedavisi alan 200 hasta prospektif olarak değerlendirilmiştir. 23-49 yaşları arasında regüler menstrüel sikluslaı olan ve FSH değerleri 12 mIU/ml altında olan hastalar çalı şmaya dahil edilmiştir. Bazal dönemde FSH, LH, E2, PRL, TSH, T ve SHBG seviyeleri saptanmıştır. Serbest androjen indeksi (SAI) hesaplanmıştır. IVF stimülasyon parametreleri, fertilizasyon ve klinik gebelik oranları değerlendirilmiştir.

BULGULAR: Sigara öyküsü T'de artış ile ilişkili bulunmuştur. Bazal T ile toplanan oosit sayısı ve transfer edilen embriyo sayısı arasında anlamlı pozitif korelasyon saptanmıştır. Ancak bazal T ve SHBG seviyeleri ile fertilizasyon ve klinik gebelik oranları arasında ilişki saptanmamıştır.

SONUÇ: Bazal serum T seviyeleri ile toplanan oosit sayısı ve transfer edilen embriyo sayısı arasında pozitif korelasyon saptanmıştır. Ancak T ve SHBG seviyeleri ile fertilizasyon ve gebelik oranları arasında ilişki saptanmamıştır.

Anahtar Kelimeler: Testosteron, SHBG, Bazal hormonlar, IVF parametreleri, Gebelik sonucu

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