

Effect of Basal Testosterone and Dehydroepiandrosterone Sulphate Levels on in Vitro Fertilization Outcome in Normoresponders

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OBJECTIVE: The purpose of the present study is to evaluate the predictive value of basal total testosterone (total-T) and dehydroepiandrosterone sulphate (DHEAS) levels in normoresponder patients undergoing IVF-ET.

STUDY DESIGN: A total of 60 normoresponder infertile women, scheduled to undergo their first IVF/ET cycle were enrolled in this study. Day 2-4 basal concentrations of FSH, LH, E₂, total testosterone, DHEAS and antral follicle count (AFC) were determined in preceding cycle.

RESULTS: No significant difference was found between pregnant and non-pregnant groups regarding the AFC, hormone and controlled ovarian hyperstimulation (COH) parameters (total dose of gonadotrophins (1995.7±851.1 vs 2296.4±845.3 IU), >14 mm follicles (9.3±3.1 vs 7.3±3.9), retrieved oocytes (12.4±5.1 vs 8.7±6.1) or number of good quality embryos (7.4±3.7 vs 4.4±3.6) (p>0.05). Testosterone and DHEAS was not correlated with COH parameters (p>0.05).

CONCLUSION: There is no correlation between testosterone and DHEAS levels and COH parameters in normoresponder IVF patients.

Key Words: Androgen, Normoresponder, In vitro fertilization

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Introduction

Prediction of response in in vitro fertilization (IVF) is still a challenge to the clinician. Being an invasive, stressful, time-consuming and expensive treatment makes the prediction of response vital prior to treatment cycle to prevent disappointment and distress of the patients as well as optimizing the gonadotropins stimulation protocol to obtain a good response. To date, many a number of parameters have been used to predict the pregnancy. The predictive value of many widely used parameters as age, baseline tests like follicle stimulating hormone (FSH), Inhibin B, estradiol (E₂) and anti-Mullerian hormone (AMH) and antral follicle count (AFC) is still seem to

be not accurate enough.¹ The tests are specific for prediction of decreased ovarian reserve but not sensitive, which led many investigators to evaluate other potential markers to identify patients that will have successful pregnancy outcome.

Androgens, primarily testosterone (T), play an important role in follicular recruitment. T enhances follicular growth via increasing FSH receptor activity and stimulating insulin-like growth factor-I (IGF-I). Recent clinical reports with encouraging results demonstrated that cotreatment with androgen, such as dehydroepiandrosterone (DHEA) and transdermal testosterone, could increase both quantity and quality of oocytes and embryos, and improve pregnancy outcomes in women with diminished ovarian function or even premature ovarian failure.²⁻⁵

The purpose of the present study is to evaluate the predictive value of basal total testosterone (Total-T) and dehydroepiandrosterone sulfate (DHEAS) levels in normoresponder patients undergoing IVF-ET with the indication of unexplained and male infertility.

Material and Method

Participants

A total of 60 women scheduled to undergo their first intra-

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cytoplasmic sperm injection (ICSI)/embryo transfer (ET) cycle with the indications of male infertility or unexplained infertility in our infertility and IVF unit between April 2012 and October 2012 were enrolled in this study. Thirty were treated with long luteal GnRH agonist protocol and 30 patients were treated with flexible multidose GnRH antagonist protocol. Exclusion criteria were women with biochemical and/or ultrasonographic evidence of polycystic ovarian syndrome (PCOS), endometriosis, basal FSH value >12 IU/L, age >40, history of ovarian surgery, chromosomal abnormalities, endocrinological and/or autoimmune disorders. The baseline of serum concentration of FSH, luteinizing hormone (LH), estradiol (E₂), Total-T and DHEAS were determined for each patient on the 2nd-4th day of the unstimulated cycle preceding IVF treatment. Serum levels of FSH, LH, E₂, DHEAS, Total-T were measured with electrochemiluminescence assays (ELECYS 2010 HITACHI, Roche Diagnostic, Germany). Ultrasonographic evaluation was done on the same day with basal endocrine evaluation to determine the antral follicle count (AFC). The study was approved by Ethics Committee and written informed consent was obtained from all participants.

Stimulation Protocols

In patients who underwent a GnRH agonist long luteal down-regulation protocol, pituitary desensitization was induced with the administration of the GnRH agonist leuprolide acetate (Lucrin, Abbot, Turkey) in the midluteal phase of the previous cycle until the day of HCG administration. After onset of menstrual bleeding, when satisfactory pituitary desensitization was achieved (serum E₂ level <50 pg/ml, endometrial thickness <5 mm, serum LH levels <5 IU/ml), GnRH agonist dose is reduced to half and gonadotrophin administration was started. Women with delayed suppression (including subjects who developed ovarian cysts after the GnRH agonist administration) were excluded from the study. Patients who were treated with antagonist protocol received recombinant FSH starting on day 3 and 0.25 mg cetorelix (Cetrotide; Asta Medica, Frankfurt, Germany) administered daily when two or more follicles reached 13-14 mm in diameter. Gonadotropin stimulation was started with a daily use of recombinant FSH (Gonal-F; Merck Serono, Istanbul, Turkey or Puregon, Organon, Istanbul, Turkey). The dose of gonadotropins was adjusted according to ovarian response determined by follicular growth monitored by transvaginal ultrasonography and serum E₂ concentrations. Recombinant hCG (250 micrograms sc., Ovitrelle, Serono, Istanbul, Turkey) was administered when at least three follicles showed a mean diameter of 17 mm. Oocytes were retrieved by transvaginal ultrasound-guided aspiration 35 h after the hCG injection. Following oocyte retrieval, metaphase II oocytes were reviewed and good-quality embryos were transferred under ul-

trasonographic guidance on day 2 or 3 for all patients. Only one embryo was transferred for women aged <35 years old and two embryos were transferred for women aged ≥ 35 years old. All patients received vaginal progesterone (Crinone 8% gel, Serono) supplementation twice a day until 12 weeks of gestation. Clinical pregnancy was defined as the presence of a gestational sac with accompanying fetal heartbeat by ultrasound 4 weeks following the ET procedure.

Statistical analysis

Statistical analysis was performed by using IBM SPSS Statistics Software (19.0, SPSS Inc., Chicago, IL, USA). Whether the distributions of continuous variables were normal or not, was determined by the Kolmogorov-Smirnov test. The parametric results were presented as mean ± standard deviation values and compared by using the Independent Samples t test when distributed normal. Mann-Whitney U test was used when the results were not found to be distributed normal or for comparison of nonparametric data. Categorical variables were compared with Fisher's exact or Pearson chi-square tests when available. P values <0.05 were considered statistically significant.

Results

The mean age and body mass index (BMI) of the participants were 29.9±4.3 and 25.7±4.4 kg/m² respectively. Among the hormone parameters mean FSH and LH values were 6.7±1.7 and 4.9±1.8 while Total-T and DHEAS levels were 32.9±24.8 and 164.1±72.3. Comparison of baseline characteristics, ultrasonographic data, hormone profiles and COH parameters of pregnant and non-pregnant patients were shown in table 1. No significant difference was found between pregnant and non-pregnant groups regarding the mean age, BMI and AFC (p>0.05). When hormone parameters (FSH, LH, E₂, Total-T, DHEAS) were compared, no significant difference were found between cases pregnant and non-pregnant (p>0.05). Regarding the COH parameters, the groups had no significant difference in total dose of gonadotrophins (1995,7 ±851.1 vs 2296.4±845.3 IU), >14 mm follicles (9.3±3.1 vs 7.3±3.9), retrieved oocytes (12.4 ±5.1 vs 8.7±6.1) or number of good quality embryos (7.4±3.7 vs 4.4±3.6) (p>0.005).

According to Pearson Correlation analysis, testosterone was not correlated with multiple stimulation parameters (total dose of gonadotrophins, number of oocytes retrieved, mature oocytes and number of good quality embryos) (p>0.05) (Table 2). A weak but statistically not significant, although very close, positive correlation between testosterone and pregnancy was found (r=0.247, p=0.057). DHEAS was also not correlated with previously mentioned four stimulation parameter (p>0.05) (Table 3).

Table 1: The comparison of characteristics and controlled ovarian hyperstimulation parameters of pregnant and nonpregnant cases

	Pregnant (n=20)	Non-Pregnant (n=40)	p values
Age (years)	29.7± 4.4	30.1±4.3	0.641
BMI (kg/m ²)	24.9±4.7	26.1±4.3	0.463
AFC	11.2±2.9	10.3±3.7	0.103
FSH	6.5±0.8	6.9 ±2.0	0.520
LH	5.1±1.9	4.9±1.8	0.938
E2	29.5±15.1	47.8±25.7	0.090
Total-T	41.7±25.3	28.5±23.7	0.827
DHEAS	150.4±80.7	170.9±67.8	0.354
Total dose of gonadotrophins	1995.7±851.0	2296.4±845.3	0.344
Induction days	10.1±0.8	10.1±1.2	0.139
E2 day of hCG	2439.9±794.8	1994.6±1062.7	0.096
P on day of hCG	0.8±0.3	0.9±0.5	0.103
>14 mm follicle	9.3±3.1	7.3±3.9	0.247
Endometrial thickness	10.2±1.3	10.2±1.3	0.412
Retrieved oocytes	12.4±5.1	8.7±6.1	0.282
Metaphase II oocytes	10.8±4.6	7.0±4.9	0.409
2PN	7.6±3.8	4.7±3.7	0.747
Number of good quality embryos	7.4±3.7	4.4±3.6	0.741
Number of embryos transfered	1.2±0.4	1.2±0.9	0.460

AFC: Antral follicle count, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, Total-T: Total testosterone, DHEAS: Dehydroepiandrosterone sulfate, hCG: Human chorionic gonadotrophin

Table 2: Spearman correlation analysis of the variables with total testosterone values

Variables	Correlation coefficient	p values
Number of oocytes retrieved	0.157	0.229
Total dose of gonadotrophins (IU)	-.002	0.986
Number of good quality embryos	0.139	0.288
Pregnancy	0.247	0.057

Table 3: Spearman correlation analysis of the variables with DHEAS values

Variables	Correlation coefficient	p values
Number of oocytes retrieved	0.024	0.853
Total dose of gonadotrophins (IU)	-0.085	0.518
Number of good quality embryos	-0.014	0.916
Pregnancy	-0.159	0.224

Discussion

The results of this study demonstrated that there was no

statistically significant correlation between testosterone, DHEAS levels and IVF stimulation parameters and pregnancy outcome.

It is well established that androgens and androgen receptors (AR) are important for normal follicular growth and progression beyond preantral stage.⁶ Androgens can directly influence ovarian follicle development by local intra-ovarian androgen receptor mediated actions,^{7,8} augmentation of the growth by enhancing effect of IGF-I and augmentation of granulosa cell FSH receptor expression.⁹ Inhibition of ARs slows mouse follicle growth,¹⁰ decreases the diameter of mouse follicles^{11,12} and stimulates granulosa cell degeneration and follicular apoptosis.¹³ It's assumed that there is an age related decrease in testosterone secretion from theca cells similar to AFC which causes a progressive impairment of the aging ovary's ability to respond to stimulation for fertility treatments. This relation between decreasing androgens and impaired response to stimulation which is the situation seen in poor responders is studied previously by many authors in literature. Increasing intraovarian androgens by means of DHEA or by aromatase inhibitors makes the intraovarian hormonal milieu appropriate for follicular growth, improve oocyte and embryo quality in poor responders.¹⁴ In a retrospective study, Qin et al reported that basal T levels were sig-

nificantly higher in the pregnant women than that in non-pregnant ones in poor responder group also ($p<0.05$).¹³

The reports on normoresponders are limited in the number and our study support previous studies reporting lack of correlation between basal androgen levels and pregnancy outcomes in normoresponder patients.^{13,15} Frattarelli et al. evaluated androgen levels to determine their predictive value to the IVF cycle parameters and/or cycle outcome and suggested that basal testosterone levels ≤ 20 ng/dL was associated with poor IVF success rates.¹⁶ But later, the same group denied the predictive value and their prospective data do not support the findings from the prior retrospective analysis. They did not find a significant androgen level at which pregnancy outcome was better. Neither baseline T and androstenedione nor DHEAS had a significant correlation with any stimulation parameter.¹⁵ In our study we also could not find a significant correlation between testosterone levels (although very close to significance), DHEAS levels and pregnancy ($r=0.247$, $p=0.057$ and $r=-0.159$, $p=0.224$ respectively). Qin et al also showed that the basal T could predict the pregnancy outcome in women with decreased ovarian reserve, but they could not find the same association with normal ovarian reserve group.¹³ In our study, which is also performed on normoresponder population, no significant difference in basal testosterone and DHEAS levels were found between pregnant and non-pregnant groups either. On the contrary to poor responders, in whom basal T levels were significantly higher in the pregnant women than that in non-pregnant ones, higher androgen levels may also be detrimental in normoresponders. In a prospective study conducted on 103 normoresponder patients, priming with aromatase inhibitors and hCG was compared to standard antagonist protocol but the number of top quality embryos, the implantation rate, the pregnancy rate and the delivery rate was not improved in the priming group compared to the control group.¹⁷

A weakness of this study is the limited number of the sample size. But evaluation of the ovarian androgen, basal serum testosterone and DHEAS, adrenal androgens in the same study is the strength of this study.

In summary, basal serum testosterone and DHEAS levels did not show a correlation with total dose of gonadotrophins, number of oocytes retrieved, mature oocytes and number of good quality embryos in normoresponder patients. But the relation with testosterone and pregnancy outcome should be clarified with further large scale studies.

Normoresponder Hastalarda Bazal Testosteron ve Dehidroepiandrosteron Sülfat Düzeylerinin In Vitro Fertilizasyon Sonuçlarına Etkisi

AMAÇ: Bu çalışmanın amacı normoresponder hastalarda bazal androjen düzeylerinin IVF sonuçlarına etkisini değerlendirmektir.

GEREÇ VE YÖNTEM: İlk IVF/ET denemesi için kabul edilen 60 normoresponder hastanın siklusun 2-4. günlerinde bazal serum FSH, LH, E₂, Total-Testosteron (total-T), Dehidroepiandrosteron sülfat (DHEAS) düzeyleri ve antral follikül sayıları (AFS) değerlendirildi.

BULGULAR: Gebe kalan ve kalamayan hastalar karşılaştırıldığında AFS, hormon ve kontrollü ovaryan hiperstimülasyon (KOH) parametreleri (kullanılan total gonadotropin miktarı (1995,7±851,1 vs 2296,4±845,3 IU), 14 mm üzerinde follikül sayısı (9,3±3,1 vs 7,3±3,9), elde edilen oosit sayısı (12,4±5,1 vs 8,7±6,1) ve iyi kalitede embriyo sayıları (7,4±3,7 vs 4,4±3,6) arasında istatistiksel olarak anlamlı fark saptanmadı ($p>0,005$).

Testosteronun ve DHEAS ile KOH parametreleri arasında istatistiksel olarak anlamlı korelasyon saptanmadı ($p>0,05$).

SONUÇ: Normoresponder hasta grubunda bazal testosteron ve DHEAS düzeyleri ile IVF stimülasyon parametreleri arasında korelasyon yoktur.

Anahtar Kelimeler: Androjen, İn vitro fertilizasyon, Normoresponder

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