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), total testosterone, DHEAS and antral follicle count (AFC) are still seem to be not accurate enough. 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.

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.
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 patients 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 (ELECSYS 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, onset of menstrual bleeding, when satisfactory 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 cetrorelix (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 ultrasonographic 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 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 2: Spearman correlation analysis of the variables with total testosterone values

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation coefficient</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of oocytes retrieved</td>
<td>0.157</td>
<td>0.229</td>
</tr>
<tr>
<td>Total dose of gonadotrophins (IU)</td>
<td>-0.002</td>
<td>0.986</td>
</tr>
<tr>
<td>Number of good quality embryos</td>
<td>0.139</td>
<td>0.288</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>0.247</td>
<td>0.057</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation coefficient</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of oocytes retrieved</td>
<td>0.024</td>
<td>0.853</td>
</tr>
<tr>
<td>Total dose of gonadotrophins (IU)</td>
<td>-0.085</td>
<td>0.518</td>
</tr>
<tr>
<td>Number of good quality embryos</td>
<td>-0.014</td>
<td>0.916</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>-0.159</td>
<td>0.224</td>
</tr>
</tbody>
</table>

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. Androgens can directly influence ovarian follicle development by local intra-ovarian androgen receptor mediated actions, augmentation of the growth by enhancing effect of IGF-I and augmentation of granulosa cell FSH receptor expression. Inhibition of ARs slows mouse follicle growth, decreases the diameter of mouse follicles and stimulates granulosa cell degeneration and follicular apoptosis. 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.
normoresonder hastalarda bazal androjen düzeylerinin IVF sonuçlarına etkisini değerlendirilmek.

**BULGULAR:** Gebe kalan ve kalamayan hastalar karşılaştırıldığında AFS, hormon ve kontrollü ovarian hiperstimülasyon (KOH) parametreleri (kullanılan total gonadotropin miktarı (1995,7±851,1 vs 2296,4±845,3 IU), 14 mm üzerinde folikül 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 sıtaplandı (p<0,005).

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

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

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 oocyte 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.

**Normoresonder Hastalarda Bazal Testosteron ve Dehidroepiandrosteron Sülfat Düzeylerinin in Vitro Fertilizasyon Sonuçlarına Etkisi**

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**References**


