Effects of exogenous testosterone supplementation in gonadotrophin stimulated cycles

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BACKGROUND: Various experiments suggest that ovarian follicular recruitment and growth may be increased by testosterone priming. Our aim was to determine the effects of exogenous testosterone supplementation in older women on ovarian folliculogenesis and steroidogenesis.

METHODS: A prospective randomized double-blind placebo-controlled crossover study was carried out. Twelve regularly menstruating non-obese women aged 38–45 years received a 12-day course of transdermal testosterone (2.5 mg per patch) or placebo patch, followed by 7 days of gonadotrophin stimulation. After at least a 1 month washout period, subjects underwent the same protocol using the opposite treatment. The main outcomes were follicular development (ultrasound measures) and hormone levels.

RESULTS: Following gonadotrophin stimulation, there were no differences in average number of follicles over 10 mm diameter in cycles pre-treated with testosterone versus placebo [2.10 (95% confidence interval (CI) 1.11, 3.22) versus 2.08 (95% CI 1.03, 3.14), P = 0.55]. No crossover, period (first or second test) or sequence (order of treatment) effects were noted. As expected, total and free testosterone levels were increased following testosterone treatment (312.7 ± 122.4 versus 12.3 ± 4.5 ng/dl and 45.5 ± 16.7 versus 1.4 ± 0.5 ng/dl, respectively, P < 0.001) but no differences in free or total testosterone were noted by period. LH, FSH, estradiol and antral follicle counts before gonadotrophin stimulation were not altered by testosterone pretreatment or by period.

CONCLUSIONS: Despite increased testosterone levels, a short course of androgens had no significant effect on the number of follicles over 10 mm during stimulation with FSH in women of late reproductive age.

Key words: testosterone / IVF / gonadotrophins / ovary / oocytes

Introduction

Success with controlled ovarian hyperstimulation (COH) depends on the ability to recruit adequate numbers of follicles. Unfortunately, some patients produce too few follicles with COH and are classified as poor responders. Although the definition of what constitutes a poor response varies widely (Surrey and Schoolcraft, 2000), the incidence in a population undergoing assisted reproduction treatment is estimated to be 9–24% (Fasouliotis et al., 2000; Shanbhag et al., 2007), thus representing a clinically important problem.

Over the years, numerous techniques and therapies have been developed in an effort to help the poor responder, but few have met with success (Kucuk et al., 2008; Kyrou et al., 2009). Primate studies, however, suggest that androgens may play a role in folliculogenesis and consequently could be used as a potential therapeutic modality. For example, short-term administration of testosterone in rhesus monkeys increases the number of pre-antral follicles, decreases ovarian follicle apoptosis and increases the number of theca and granulosa cell populations in ovarian follicles when compared with placebo (Vendola et al., 1998). These findings are reproducible when testosterone is replaced with dihydrotestosterone suggesting that the mechanism of action is via the androgen receptor and not simply through higher estradiol (E₂) levels. Additionally, exogenous androgen administration for 10 days has been shown to increase the mRNA of the FSH receptor by 50–100% in the rhesus monkey (Weil et al., 1999).

Androgens have also been shown to enhance FSH-induced hormone production from cultured granulosa cells in the primate
underwent the same protocol using the opposite treatment (placebo by androgen therapy. Medroxyprogesterone (10 mg daily) was taken to demonstrate subtle changes in sensitivity to FSH that might be induced by androgen therapy. The placebo patch was identical to the testosterone patch, matching in size and color. Patches underwent a baseline transvaginal ultrasound (TVUS) and blood draw. On day 8 of the cycle, the first TVUS and blood draw was performed. This dose and duration of Follistim treatment was chosen in our agreement with the Food and Drug Administration (FDA) and the American Society for Reproductive Medicine (ASRM). On the morning of removal, another TVUS and blood draw was performed. Randomization was achieved by a third party using a computerized random number generator with permuted blocks of 10. Upon entry into the trial, women were allocated the next available number on the list. Subjects were recruited to participate via advertising in the daily hospital newsletter between March 2006 and May 2007. Inclusion criteria were regular menstruation (defined as a cycle interval of 22–35 days for the last 3 months), age 38–45 years, presence of two ovaries and a uterus, absence of hormonal medication use and willingness to use two forms of non-hormonal contraception for the duration of the study. Women with polycystic ovary syndrome, signs of hyperandrogenism, obesity (BMI > 30 kg/m²), smokers and those attempting to conceive were excluded from the study. Subjects were recruited to participate via advertising in the daily hospital newsletter between March 2006 and May 2007. Appropriate subjects gave informed consent and then were randomly assigned to start treatment. Randomization was achieved by a third party using a computerized random number generator with permuted blocks of 10. Upon entry into the trial, women were allocated the next available number on the list. Patches, both placebo and Androderm (testosterone patch), were pre-packaged in groups of 12 and distributed at the Clinical Research Center by the third party according to the patient’s number. Study personnel and participants were blinded to treatment assignment for the duration of the study. The code was revealed to the researchers only after recruitment, data collection, and laboratory analyses were complete. Participants were compensated financially upon completion of the study. Between cycle Days 1–4 of a spontaneous menstrual period, participants underwent a baseline transvaginal ultrasound (TVUS) and blood draw for hormone measurements including FSH, LH, total/free testosterone and E2. Starting on cycle Day 16, a new patch (either placebo or testosterone 2.5 mg) was applied to the skin each evening. The placebo patch appeared identical to the testosterone patch, matching in size and color. The patches were worn for a total of 12 days (during the luteal phase of the cycle) and then removed. On the morning of removal, another TVUS and set of blood samples were obtained and gonadotrophin stimulation was begun immediately afterwards whether or not menstrual bleeding had occurred. Patients received a 7-day course of Follistim (Organon Inc) 75 IU/day and on cycle Day 8, the third TVUS and blood draw was performed. This dose and duration of Follistim treatment was chosen in an effort to minimize the risks of ovarian hyperstimulation in these subjects. We also felt that this relatively lower dose was more likely to demonstrate subtle changes in sensitivity to FSH that might be induced by androgen therapy. Medroxyprogesterone (10 mg daily) was taken orally to induce a menses at the end of the stimulation. Following at least a 1-month washout period and spontaneous menses, the subjects underwent the same protocol using the opposite treatment. All hormone assays were performed at a single laboratory and all ultrasounds were performed within the division by personnel blinded to the treatment arm. Participants were asked to keep a daily symptoms log and physical exams were conducted at each visit focusing on the presence of acne and hirsutism.

Materials and Methods

We conducted a prospective randomized double-blind placebo-controlled crossover study following Institutional Review Board approval. Inclusion criteria were regular menstruation (defined as a cycle interval of 22–35 days for the last 3 months), age 38–45 years, presence of two ovaries and a uterus, absence of hormonal medication use and willingness to use two forms of non-hormonal contraception for the duration of the study. Women with polycystic ovary syndrome, signs of hyperandrogenism, obesity (BMI > 30 kg/m²), smokers and those attempting to conceive were excluded from the study. Subjects were recruited to participate via advertising in the daily hospital newsletter between March 2006 and May 2007. Appropriate subjects gave informed consent and then were randomly assigned to start treatment. Randomization was achieved by a third party using a computerized random number generator with permuted blocks of 10. Upon entry into the trial, women were allocated the next available number on the list. Patches, both placebo and Androderm (testosterone patch), were pre-packaged in groups of 12 and distributed at the Clinical Research Center by the third party according to the patient’s number. Study personnel and participants were blinded to treatment assignment for the duration of the study. The code was revealed to the researchers only after recruitment, data collection, and laboratory analyses were complete. Participants were compensated financially upon completion of the study. Between cycle Days 1–4 of a spontaneous menstrual period, participants underwent a baseline transvaginal ultrasound (TVUS) and blood draw for hormone measurements including FSH, LH, total/free testosterone and E2. Starting on cycle Day 16, a new patch (either placebo or testosterone 2.5 mg) was applied to the skin each evening. The placebo patch appeared identical to the testosterone patch, matching in size and color. The patches were worn for a total of 12 days (during the luteal phase of the cycle) and then removed. On the morning of removal, another TVUS and set of blood samples were obtained and gonadotrophin stimulation was begun immediately afterwards whether or not menstrual bleeding had occurred. Patients received a 7-day course of Follistim (Organon Inc) 75 IU/day and on cycle Day 8, the third TVUS and blood draw was performed. This dose and duration of Follistim treatment was chosen in an effort to minimize the risks of ovarian hyperstimulation in these subjects. We also felt that this relatively lower dose was more likely to demonstrate subtle changes in sensitivity to FSH that might be induced by androgen therapy. Medroxyprogesterone (10 mg daily) was taken orally to induce a menses at the end of the stimulation. Following at least a 1-month washout period and spontaneous menses, the subjects underwent the same protocol using the opposite treatment. All hormone assays were performed at a single laboratory and all ultrasounds were performed within the division by personnel blinded to the treatment arm. Participants were asked to keep a daily symptoms log and physical exams were conducted at each visit focusing on the presence of acne and hirsutism.

Statistical analysis

Our primary outcome of interest was the number of ovarian follicles > 10 mm in mean diameter that developed after gonadotrophin stimulation. A doubling in the number of follicles > 10 mm was assumed to be significant (an increase from two to four follicles with SD of 2) and we chose these numbers based on clinical observation of poor responders to gonadotrophin stimulation. This was thought to be a clinically relevant difference since many IVF programs would cancel a cycle for only two developing follicles, while four would proceed to embryo transfer. Using these parameters, 12 subjects were needed in each arm to achieve a power of 80% at the 0.05 level. Secondary outcomes measured included changes in total testosterone, free testosterone, FSH, LH and E2 levels. Differences in side effects with each treatment were also noted. Analysis was performed using Stata 9.2 (College Station, TX, USA), with crossover analysis used for the primary outcome (average total number of follicles over 10 mm after FSH stimulation). Variables included in the analysis included the following: period, designated as period 1 for the first time testing and period 2 for the second encounter; treatment, which indicated exposure to the placebo or the testosterone patch; sequence, which indicated the order in which each individual received the treatment, with sequence 1 being placebo followed by testosterone and sequence 2 indicating testosterone followed by placebo. Interactions terms included in this analysis were (treatment × period) and (treatment × sequence). Carryover was the influence of the first treatment on the outcome of the second treatment. Washout time was calculated from the first day of the stimulation in period 1 until the first day of stimulation in period 2. The remaining variables were assessed for normality and analyzed using Wilcoxon signed-rank test or paired Student’s t-test as appropriate. When Wilcoxon signed-rank was used, the analysis was repeated using paired Student t-test to evaluate the influence of the non-normal distribution pattern on the outcome. If results were similar, distribution was assumed to have minimal influence and paired Student t-test results were reported.

Results

A total of 67 women responded to our advertisements and 28 met the pre-set eligibility criteria for the study. After reviewing the study in more detail and discussing the consents, 13 agreed to participate (Fig. 1). These 13 women underwent randomization and 12 of the 13 completed the trial. One subject was withdrawn for an abnormal finding on the initial ultrasound. At study entry, the participating subjects had a mean (± SD) age of 42.3 ± 2.7 years (range 38.3–46.2), mean BMI of 24.6 ± 3.8 kg/m², a mean FSH of 8.0 ± 4.1 mIU/ml and an average cycle interval of 27.1 ± 3.2 days. Bivariate analyses of these variables by intended treatment arm were not significantly different (Table I).

Six women were treated with sequence 1 and six were treated with sequence 2. The average time of washout was 92.6 ± 33.4 days. Women receiving sequence 1 had an average washout of 86.3 ± 24.4 days and those receiving sequence 2 had an average washout of 98.8 ± 42.0 days (P = 0.54).
No difference was seen in the average total number of follicles over 10 mm after FSH stimulation between placebo and testosterone treatment [2.08 (95% confidence interval (CI) 1.03, 3.14) versus 2.10 (95% CI 1.11, 3.22), respectively, \( P = 0.55 \)] (Fig. 2). No effect was seen for carryover (\( P = 0.19 \)), period (\( P = 0.15 \)) or sequence (\( P = 0.33 \)), and no interactions were found between (treatment × period) (\( P = 0.19 \)). There was a significant interaction between (treatment × sequence) (\( P = 0.005 \)), but this did not change overall significance.

Total number of follicles for each subject by treatment and period are shown in Fig. 3. There was also no difference in serum E₂ levels after FSH stimulation when comparing cycles pre-treated with testosterone versus those with placebo (262.7 ± 301.6 versus 318.4 ± 285.9 pg/ml, respectively, \( P = 0.8 \)).

Subgroup analysis was performed to determine development of the follicles when pretreated with testosterone. Subgroups included follicles <10 mm, follicles 11–15 mm, follicles 16–18 mm and follicles

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**Figure 1** Consort flowchart.
>18 mm and no differences were seen in these groups (Fig. 4) (P = 0.9, 0.57, 0.65 and 0.28, respectively).

As would be expected, total and free testosterone levels were significantly increased with testosterone treatment compared with placebo, indicating that treatment was successful in raising testosterone levels. No significant differences were found between total or free testosterone levels measured prior to FSH stimulation by period of administration, or total testosterone measure on cycle Day 8 by period (Table II). Testosterone treatment was well tolerated, and no significant differences in side effects, including acne and hirsutism, were noted. There were no significant differences in the second measurements (prior to FSH stimulation) of LH, FSH, E2 or antral follicle count when compared between treatments or periods (Table II).

**Discussion**

These data indicate that a short (12 day) course of testosterone administration resulted in no differences in the total number of follicles that develop to a size >10 mm in response to FSH stimulation, despite significantly elevated serum testosterone levels. Although the dose and duration of FSH treatment was low at 75 IU/day for 7 days, this treatment regimen was chosen to minimize the risks of hyperstimulation in these subjects. We also felt that this relatively lower dose was more likely to demonstrate subtle changes in sensitivity to FSH that might be induced by androgen therapy. The dose and duration of testosterone administration was based on primate
studies (Vendola et al., 1998; Weil et al., 1999) suggesting improved follicular numbers and development with a similar exposure. It should be noted that by cycle Day 8 following FSH stimulation, testosterone levels were similar in the two groups despite significantly higher testosterone levels at the start of the cycle in women pre-treated with testosterone. It remains possible that different exposures (dose or duration) to testosterone could prove beneficial to follicular development in women. We have previously reported improved ovarian response to gonadotrophin therapy with testosterone pretreatment in a woman with Kallmann’s Syndrome (Sipe et al., 2007). There may be other subgroups of infertile women that would show an improved response to testosterone treatment.

Clinical observations suggest that either endogenous or exogenous androgens can lead to increased sensitivity to gonadotrophin stimulation. Elevated intraovarian testosterone levels may be the mechanism behind the increase in small antral follicles seen histologically following oophorectomy (Futterweit and Deligdisch., 1986; Spinder et al., 1989; Pache et al., 1991a, b).

Low levels of endogenous androgens (testosterone and dehydroepiandrosterone sulfate) have been shown to be associated with both reduced responsiveness to gonadotrophins and reduced pregnancy rates following IVF cycles (Frattarelli and Peterson, 2004). Thus it is possible that androgens affect not only follicular response to gonadotrophins but also egg and embryo quality-outcomes we could not assess in this study.

Several investigators have studied the effects of exogenous androgens on follicular response and pregnancy rates following COH and IVF. In a case series of five women with a history of poor response to COH, use of oral micronized dehydroepiandrosterone (DHEA) for 2 months seemed to be associated with increased E2 concentrations and follicle numbers following another COH cycle (Casson et al., 2000). Prolonged use of oral DHEA has also been reported to improve IVF cycle outcomes, including ovarian responsiveness, oocyte quality and pregnancy rates, in older women or women with reduced ovarian reserve (Barad and Gleicher, 2005a, b; Barad et al., 2005, 2007). All of these studies suffer from the lack of a control group, limiting the conclusions that can be reached. In addition, findings cannot be directly compared with our study because of the use of a relatively weak androgen for a longer period of time.

The effect of exogenous testosterone on follicular response to gonadotrophin stimulation during an IVF cycle has been the subject of a few studies, with mixed results. In one study, women with a diagnosis of reduced ovarian reserve were randomized to either 1 g of testosterone gel given daily or placebo for 15–20 days prior to an IVF cycle (Massin et al., 2006). Similar to our findings, there was no difference in antral follicle count or in the number of follicles, eggs or embryos following gonadotrophin stimulation despite a significant increase in serum testosterone levels in women receiving the testosterone gel. In contrast, the use of the testosterone patch (Androderm patch, 2.5 mg/day, left on for a varying amount of time over the course of a 24-h day depending on the patient’s weight) for 5 days preceding gonadotrophin stimulation was reported to improve cycle outcomes in women with a history of two poor responses to gonadotrophins in prior IVF cycles, despite normal basal FSH levels (Balasch et al., 2006); as compared with the prior two poor responses, pretreatment with testosterone was said to be associated with a 5-fold increase in the number of recruited follicles in 80% of the participants. The non-randomized nature of this study makes it difficult to determine if this was a testosterone effect or simply regression to the mean among these women. Finally, a recent study concluded that

### Table II Data obtained after patches removed, prior to FSH stimulation (second data collection point), at cycle Day 8 and analyzed according to treatment (top panel) and period (lower panel).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Testosterone</th>
<th>Placebo</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Total testosterone (ng/dl)</td>
<td>312.7 ± 122.4</td>
<td>12.3 ± 4.5</td>
<td>&lt;0.001</td>
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<td>Free testosterone (ng/dl)</td>
<td>45.5 ± 16.7</td>
<td>1.4 ± 0.5</td>
<td>&lt;0.001</td>
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<tr>
<td>FSH (mIU/ml)</td>
<td>8.6 ± 6.0</td>
<td>9.8 ± 9.9</td>
<td>0.7</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>5.4 ± 3.1</td>
<td>5.4 ± 3.1</td>
<td>0.99</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>34.4 ± 13.4</td>
<td>39.3 ± 23.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Antral Follicle Count</td>
<td>12.9 ± 7.6</td>
<td>11.75 ± 8.5</td>
<td>0.73</td>
</tr>
<tr>
<td>Total testosterone, cycle Day 8 (ng/dl)</td>
<td>18.7 ± 8.5</td>
<td>18.7 ± 8.3</td>
<td>0.99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Period 1</th>
<th>Period 2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total testosterone (ng/dl)</td>
<td>149.2 ± 157.7</td>
<td>175.75 ± 197.5</td>
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<tr>
<td>Free testosterone (ng/dl)</td>
<td>19.4 ± 20.0</td>
<td>27.49 ± 30.0</td>
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<tr>
<td>FSH (mIU/ml)</td>
<td>8.8 ± 6.0</td>
<td>9.6 ± 9.6</td>
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<tr>
<td>LH (mIU/ml)</td>
<td>5.4 ± 3.4</td>
<td>5.3 ± 2.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>34.3 ± 14.7</td>
<td>39.4 ± 22.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Antral follicle count</td>
<td>12.8 ± 7.8</td>
<td>11.9 ± 8.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Total testosterone, cycle Day 8 (ng/dl)</td>
<td>18.9 ± 8.5</td>
<td>18.5 ± 8.4</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD.
pretreatment with transdermal testosterone (Androderm patch, 2.5 mg per day for 5 days) improved the ovarian sensitivity to gonadotrophin stimulation in a population of women with a history of a low response in an IVF cycle (Fábregues et al., 2009). Women were randomized to testosterone and a standard stimulation protocol versus no testosterone and a high dose gonadotrophin protocol. Thus, a second variable (gonadotrophin regimen) was introduced in addition to testosterone use, complicating interpretation of results. Nevertheless, testosterone use was associated with a trend towards a lower cycle cancellation rate (19.4 versus 41.9%, \( P = 0.09 \)) although there was no difference in number of eggs retrieved, number of embryos or pregnancy rates.

Strengths of our study include the prospective, randomized, crossover design that allowed each woman to serve as her own control. The closely spaced stimulations helped to minimize the effect of age on response in these women, which is an important consideration when dealing with women toward the end of their functional reproductive lifespan. Our study had sufficient power to detect a clinically significant difference with the use of testosterone; however, it is possible that some of these women had ovarian reserves that were so low that they would not have responded to any stimulation protocol and this may have biased the results toward the null. If this is true, then younger women or women with greater ovarian reserves given the same protocol might have a more robust response to testosterone pretreatment. Therefore this study is limited because it cannot be generalized to all women of reproductive age.

Despite suggestions from animal studies of a beneficial effect of short-term exogenous testosterone administration on gonadotrophin responsiveness, our results and those in the literature suggest there is either no effect or only a subtle effect of this therapy in women with poor responders undergoing assisted reproductive technology: a challenge to meet.

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