

# Comparison of androgen levels in conception vs. non-conception cycles following controlled ovarian stimulation using the luteal phase gonadotropin-releasing hormone agonist protocol

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## ABSTRACT

Serum concentrations of androstenedione, testosterone and dehydroepiandrosterone sulfate (DHEAS) as well as estradiol and progesterone were measured throughout the in vitro fertilization (IVF) cycle and compared by conception outcome to try to determine if differing levels of androgens could help elucidate the endocrine environment conducive to successful IVF cycles. The luteal phase gonadotropin-releasing hormone agonist (GnRH-a) protocol was used for ovarian stimulation. Of the 46 women enrolled in the study, 11 conceived and 35 did not conceive. Throughout the follicular phase, levels of androstenedione and DHEAS were found to rise but the same pattern of increase was found in both conception and non-conception cycles. The pattern of testosterone increase in non-conception cycles was faster than that in conception cycles. Differences in mean levels of androstenedione, testosterone, estradiol and progesterone by conception outcome in the late luteal phase can be attributed to secretion by the corpus luteum. It is possible that those women having multiple failed cycles with rapidly rising

serum testosterone levels should be considered for longer use of the GnRH-a. Differences in the pattern of testosterone rise should be monitored.

## INTRODUCTION

One explanation for failure to achieve pregnancy despite apparent ovulation induction using follicle-maturing drugs such as clomiphene citrate or human menopausal gonadotropin (hMG) is that follicle-maturing drugs may increase midcycle androgen levels<sup>1,2</sup>. The increase in androgen levels and poor pregnancy rates could also be related to basic pathological conditions such as basal hyperandrogenicity and anovulation. A recent study of patients with normal basal androgen levels found that stimulation with hMG caused an increase in androstenedione levels but this increase did not adversely affect pregnancy rates<sup>3</sup>.

The adverse effect of elevated levels of testosterone on pregnancy rates following *in vitro* fertilization-embryo transfer (IVF-ET) has been demonstrated by Laufer and co-workers<sup>4</sup> who found that cleaved oocytes associated with viable IVF pregnancies were derived from human oocyte-corona-cumulus complexes which secreted significantly less testosterone than those which did not result in a pregnancy following transfer. Other experimental data suggesting an adverse effect of testosterone were provided by Polan and associates<sup>5</sup> who found a higher concentration of testosterone in follicles stimulated by hMG than in those stimulated by follicle-stimulating hormone (FSH), leading to lower pregnancy rates in the hMG cycles (9.5% vs. 40%).

The degree of stimulation used with typical controlled ovarian hyperstimulation regimens for the purpose of stimulating oocytes for retrieval for IVF is far more excessive than for conventional stimulation for *in vivo* purposes. This is due to the increased use of gonadotropin-releasing hormone agonists (GnRH-a) to prevent premature luteinization<sup>6</sup>. In a study by Andersen and Ziebe<sup>7</sup>, serum levels of free-androstenedione and testosterone were found to be lower in the follicular phase of conceptional vs. non-conceptional cycles after ovarian stimulation using the ultrashort GnRH-a protocol. San Roman and co-workers<sup>8</sup> have also found that initiation of GnRH-a treatment in the follicular phase concurrent with the administration of hMG is associated with hyperandrogenemia and poorer pregnancy rates than with the use of luteal phase GnRH-a protocol.

The objective of this study was to compare serum levels of androgens in all phases of the cycle in women having IVF-ET treatment, using the luteal phase leuprolide acetate/hMG controlled ovarian hyperstimulation protocol, to determine if lower androgen levels are found in women achieving pregnancies vs. those who do not.

## PATIENTS AND METHODS

Forty-six women registering for their first cycle of IVF at the Cooper Center for IVF were included in this study. The women ranged in age from 26–40 years (mean  $33.0 \pm 3.9$ ). Of the women who were treated, 45.6% were admitted for tubal factor, 15.2% for male factor, 6.5% for endometriosis, 19.6% for multiple factors, 10.9% for unexplained

infertility and 2.2% for ovulatory dysfunction. There were no patients with polycystic ovarian syndrome in this group of women.

All of the women received the luteal phase leuprolide acetate/hMG hyperstimulation protocol for their IVF cycle. In this regimen, leuprolide acetate (Lupron<sup>®</sup>, TAP Pharmaceuticals, North Chicago, IL) 1.0 mg/day was administered subcutaneously for 10 days beginning on day 21 of the menstrual cycle. After 10 days of GnRH-a treatment, serum levels of estradiol and progesterone were measured. If serum estradiol was  $< 50$  pg/ml and serum progesterone was  $< 1$  ng/ml, 150 IU hMG (Pergonal<sup>®</sup>, Serono Laboratories, Randolph, MA) was administered intramuscularly twice daily. If estradiol was  $> 50$  pg/ml, 1.0 mg/day of leuprolide acetate was continued for 3–5 days and the estradiol level measurement repeated. Human menopausal gonadotropin was only initiated after ovarian suppression had been achieved. After 5 days of hMG administration, follicular development was monitored in all patients by transvaginal ultrasound and serum estradiol levels. Based on these levels, individual dosages of hMG were adjusted. When two lead follicles reached a 20-mm average diameter and the estradiol level was  $> 800$  pg/ml, the hMG administration was stopped and 10 000 IU human chorionic gonadotropin (hCG) (Profasi<sup>®</sup>, Serono Laboratories) was given intramuscularly. Leuprolide acetate was continued at 1 mg/day until the day of hCG administration. Oocyte retrieval was performed 36 h after hCG injection. Embryo transfer occurred 48 h after retrieval. Serum  $\beta$ -hCG levels were measured 14 days after embryo transfer. A clinical pregnancy was defined as ultrasound visualization of a gestational sac.

Serum levels of testosterone, androstenedione and DHEAS were measured (1) on day 10 after taking leuprolide acetate (baseline after suppression), (2) on the day of hCG injection, (3) on the day of oocyte retrieval, (4) in the midluteal phase, and (5) in the late luteal phase during the IVF cycle. Serum hormone levels of estradiol and progesterone were also measured from the day of hCG injection through to the late luteal phase. Luteinizing hormone (LH) was measured at time periods (2) and (3).

Serum specimens were assayed by solid-phase radioimmunoassay (RIA) for estradiol, DHEAS and androstenedione (Diagnostic Products Corporation, Los Angeles, CA). These methodologies

had a sensitivity of 10 pg/ml, 1.1 µg/dl and 2.0 ng/dl; intra-assay coefficient of variation (CV) of 5.6, 5.3 and 4.3% and interassay CV of 6.8, 7.0 and 6.3%, respectively. Testosterone was measured by a double-antibody RIA (ICN Biomedical Inc., Costa Mesa, CA) with a sensitivity of 4 ng/dl, intra-assay CV of 9.6% and interassay CV of 11.6%.

### Statistical analysis

Analysis of variance with repeated measures was used to compare androgen levels between patient groups and by phase of cycle. Compound symmetry of the variance-covariance matrix was tested using the Greenhouse-Geisser  $\epsilon$  test<sup>9</sup>. Adjustments to the degrees of freedom of the appropriate *F* tests were made if the assumption of compound symmetry was not met. *Post hoc* tests were used to further investigate any significant effects. A *p*-value of < 0.05 was used to determine significance.

### RESULTS

Eleven of the 46 women (24%) conceived while 35 (76%) did not conceive. There was no difference in the mean age, fertilization rate, mean number of ampules of hMG administered, or number of embryos transferred between those who conceived and those who did not (Table 1).

The mean androgen levels for the conception and non-conception cycles at each time period are presented in Table 2. Based on the analysis of variance of mean serum DHEAS levels, there was

neither a significant interaction between conception outcome and time of cycle nor was there a significant difference in mean DHEAS levels by conception outcome. For all women, however, DHEAS varied by time of cycle. There was a significant increase in mean serum DHEAS from baseline to the day of hCG injection. The levels then remained the same through to the day of retrieval, and then decreased significantly in the luteal phase. This pattern of change in DHEAS was the same in both the conception and non-conception cycles (Figure 1).

The results of the analysis of variance of mean testosterone levels showed that there was a significant interaction between conception outcome and phase of the cycle. For women who conceived, there was no difference in the mean testosterone levels through the follicular phase of the cycle, but increases were noted in the late luteal phase. For women who did not conceive, there was an increase in testosterone levels from baseline to the day of hCG injection and again from the day of hCG to the day of retrieval. The testosterone levels then decreased in the luteal phase (Figure 2).

There was also a significant interactive effect of conception outcome and phase of cycle on the mean androstenedione levels. In the conception group, androstenedione levels rose significantly from baseline to the day of hCG injection, stayed the same until the day of retrieval, but rose continuously through the luteal phase. In the non-conception group, there was a similar pattern of androstenedione rise until the day of retrieval, but

**Table 1** Comparison of *in vitro* fertilization (IVF) parameters by conception outcome. hMG, human menopausal gonadotropin

	Conception cycles (n = 11)	Non-conception cycles (n = 35)
Mean age (years)*	33.8 ± 4.0	32.8 ± 3.9
Mean number of oocytes retrieved*	13.5 ± 5.0	9.9 ± 5.2
Mean percentage fertilization*	60.3 ± 26.6	51.1 ± 24.3
Mean number of embryos transferred*	3.8 ± 1.5	3.5 ± 1.3
Mean number of ampules of hMG administered*	30.2 ± 4.3	34.1 ± 12.1
<i>Indication for IVF</i>		
Tubal factor	5 (45.4%)	16 (45.7%)
Male factor	3 (27.3%)	4 (11.4%)
Endometriosis	0	3 (8.6%)
Unexplained	0	5 (14.3%)
Ovulatory dysfunction	0	1 (2.9%)
Multiple factors	3 (27.3%)	6 (17.1%)

\*Differences between mean data for the two groups were not significant (*t*-test)

**Table 2** Comparison of androgen levels during the *in vitro* fertilization (IVF) cycle by conception outcome. Data presented as mean  $\pm$  standard deviation; DHEAS, dehydroepiandrosterone sulfate; hCG, human chorionic gonadotropin

	Phase of IVF cycle				
	Baseline (day 10 of Lupron)	Day of hCG	Day of retrieval	Midluteal phase	Late luteal phase
<i>DHEAS</i> ( $\mu\text{g}/\text{dl}$ )*					
Conception cycles	188.5 $\pm$ 107.4	205.6 $\pm$ 108.4	214.7 $\pm$ 91.9	151.7 $\pm$ 71.2	182.8 $\pm$ 72.6
Non-conception cycles	151.5 $\pm$ 66.3	185.7 $\pm$ 95.9	183.3 $\pm$ 80.1	147.7 $\pm$ 80.1	170.4 $\pm$ 85.7
<i>Testosterone</i> (ng/dl)**					
Conception cycles	30.0 $\pm$ 13.2	39.8 $\pm$ 12.9	56.0 $\pm$ 24.6	56.2 $\pm$ 21.7	79.5 $\pm$ 46.7
Non-conception cycles	30.8 $\pm$ 17.9	49.4 $\pm$ 23.3	74.6 $\pm$ 42.1	47.6 $\pm$ 31.5	25.2 $\pm$ 8.3
<i>Androstenedione</i> (ng/dl) <sup>†</sup>					
Conception cycles	197.3 $\pm$ 59.0	297.8 $\pm$ 72.1	337.7 $\pm$ 76.9	359.7 $\pm$ 104.5	495.5 $\pm$ 182.9
Non-conception cycles	175.5 $\pm$ 68.1	300.0 $\pm$ 116.0	347.1 $\pm$ 129.2	298.1 $\pm$ 143.6	156.7 $\pm$ 74.5

\*ANOVA,  $p < 0.05$  for phase effect; \*\*ANOVA,  $p < 0.05$  for interaction effect of phase and outcome;<sup>†</sup>ANOVA,  $p < 0.05$  for interaction effect of phase and outcome**Table 3** Comparison of serum hormone levels of estradiol, progesterone and luteinizing hormone (LH) during the *in vitro* fertilization (IVF) cycle by conception outcome. Data presented as mean  $\pm$  standard deviation; hCG, human chorionic gonadotropin

	Phase of IVF cycle			
	Day of hCG	Day of retrieval	Midluteal phase	Late luteal phase
<i>Estradiol</i> (pg/ml)				
Conception cycles	1450.6 $\pm$ 454.1	1230.5 $\pm$ 389.4	1298.8 $\pm$ 600.5	435.9 $\pm$ 398.5*
Non-conception cycles	1312.5 $\pm$ 555.4	1137.7 $\pm$ 565.5	972.4 $\pm$ 722.4	29.1 $\pm$ 25.3*
<i>Progesterone</i> (ng/ml)				
Conception cycles	0.98 $\pm$ 0.35	7.5 $\pm$ 3.4	103.4 $\pm$ 54.1*	64.9 $\pm$ 48.8*
Non-conception cycles	0.90 $\pm$ 0.46	6.1 $\pm$ 2.6	56.2 $\pm$ 27.6*	16.4 $\pm$ 9.1*
<i>LH</i> (mIU/ml)				
Conception cycles	15.3 $\pm$ 3.2	114.5 $\pm$ 63.7	—	—
Non-conception cycles	13.9 $\pm$ 5.1	128.1 $\pm$ 49.2	—	—

\* $p < 0.05$  comparing mean hormone levels by conception outcome at this phase of cycle

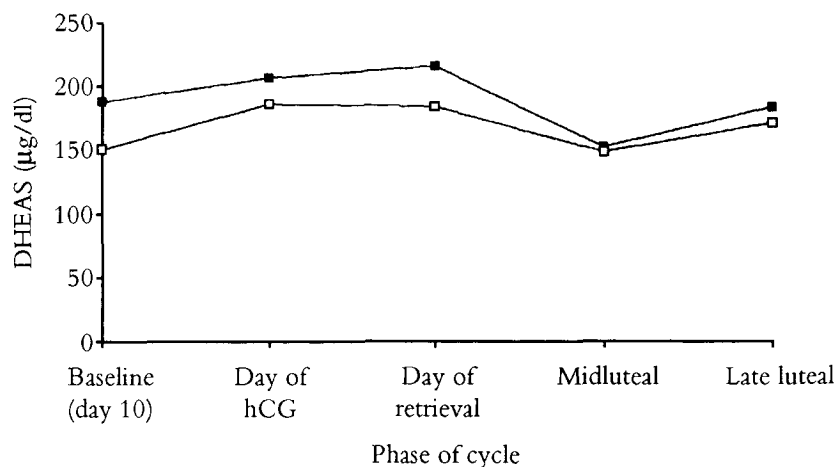
in the late luteal phase, levels decreased. There were no differences in the mean androstenedione levels by conception outcome at any time before embryo transfer (Figure 3).

The mean serum non-androgen hormone levels during the various stages of the cycle are presented in Table 3. Serum estradiol levels were the same in both outcome groups from baseline until the midluteal phase. In the late luteal phase, mean serum estradiol levels were significantly higher in the pregnant group. Similarly, mean serum progesterone levels were the same in both groups until the day of retrieval, but differed significantly

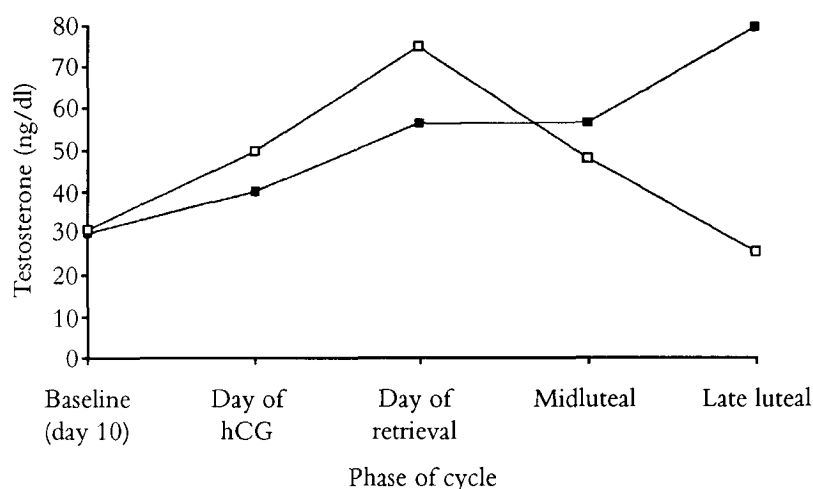
between the groups in the luteal phase. Mean serum LH levels were the same in both outcome groups.

## DISCUSSION

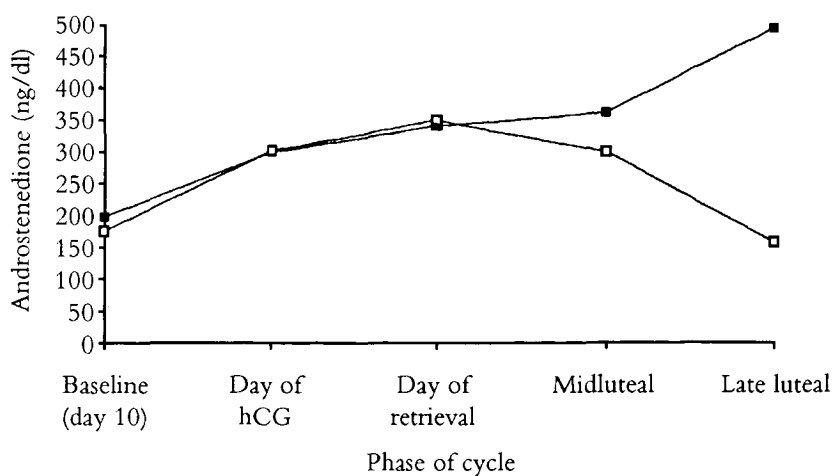
Andersen and Ziebe<sup>7</sup>, San Roman and co-workers<sup>8</sup> and Hamori and colleagues<sup>10</sup> found that hyperandrogenemia was more likely to occur when using a short GnRH-a protocol in which the GnRH-a is given concurrently with the gonadotropin therapy in the follicular phase of the cycle. The data presented in our study show that even with the



**Figure 1** Comparison of mean dehydroepiandrosterone sulfate (DHEAS) levels during the IVF cycle by outcome. Conception cycles (closed squares), non-conception cycles (open squares)



**Figure 2** Comparison of mean testosterone levels during the IVF cycle by outcome. Conception cycles (closed squares), non-conception cycles (open squares)



**Figure 3** Comparison of mean androstenedione levels during the IVF cycle by outcome. Conception cycles (closed squares), non-conception cycles (open squares)

longer use of a GnRH-a and by beginning its administration in the midluteal phase, there was an association with lower pregnancy rates and with higher serum testosterone levels on the day of hCG injection and the day of oocyte retrieval.

Although the mean levels of testosterone were not found to differ by conception outcome at day 10 after taking leuprolide acetate, the subsequent pattern of increase in testosterone differs in the two groups. In the group that conceived, mean levels of testosterone were not found to differ between baseline and the day of hCG injection whereas in the non-conception cycles, there was a steady increase in testosterone until the day of retrieval. There was a significantly higher level of serum testosterone on the day of hCG injection in those failing to conceive compared to those achieving pregnancies.

Women who conceived were found to have higher levels of estradiol, progesterone, andro-

stenedione and testosterone in the late luteal phase of the cycle – these were most likely related to the effect of rising  $\beta$ -hCG levels on corpus luteum function. The level of DHEAS, whose main source is the adrenal gland, was not found to differ by conception outcome in the late luteal phase.

Even despite the use of luteal phase leuprolide acetate for at least 10 days before using hMG, there do seem to be some patients who will have increased testosterone levels at the time of hCG injection compared to the day prior to starting hMG, and this group seems to have lower pregnancy rates. These findings should generate interest in a randomized study of patients who have rising testosterone levels at the time of hCG administration, in which one group would receive hCG but another would have the cycle cancelled and in the next cycle would take leuprolide acetate for at least double the length of time before initiating gonadotropin therapy.

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