

The effect of follicle-maturing drugs on mid-cycle androgen levels in women with normal baseline levels

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ABSTRACT

Theoretically, clomiphene citrate or human menopausal gonadotropins might have a higher chance of inducing pregnancy per cycle were it not for the concomitant rise in androgens induced by these follicle-maturing drugs. In the present study, mid-cycle androgen levels were evaluated in anovulatory women with normal baseline early follicular levels who were treated with either clomiphene citrate or human menopausal gonadotropins. The only mid-cycle androgen to rise above the normal range was androstenedione. However, no negative effects of elevated androstenedione levels on pregnancy rates were apparent. Thus, at least in women with normal baseline androgen levels, the use of follicle-maturing drugs does not appear to cause a rise in androgen levels except for androstenedione, and the rise in androstenedione at mid-cycle appears to have no adverse effect on conception.

INTRODUCTION

There is a general belief that follicle-maturing drugs, such as clomiphene citrate^{1,2} and/or human menopausal gonadotropins² may increase mid-cycle androgen levels, effecting a rise in levels

which may subsequently contribute to a lowered pregnancy rate despite apparent ovulation induction^{1,2}. These speculations were originally based on data accrued more than 25 years ago in a small population of treated patients. Whether the basic pathological condition (basal hyperandrogenicity and anovulation) decreased the pregnancy rates, or the subsequent mid-cycle elevation of androgen levels was responsible, was never made clear.

The purpose of this study was to evaluate the effect of follicle-maturing drugs on the mid-cycle androgen levels in non-hirsute, anovulatory women with normal baseline serum androgen levels, who successfully attained follicular maturation. The mid-cycle rise in the androgen, testosterone, free testosterone, androstenedione, and dehydroepiandrosterone levels, following clomiphene or human menopausal gonadotropin therapy was evaluated. Pregnancy rates were also compared in those for whom androgen levels increased above the normal range, versus patients who had levels which remained normal.

MATERIALS AND METHODS

Patients included in this study were 80 consecutive non-hirsute, anovulatory women who were treated in our infertility practice and who agreed to take part in the study. No patients were included who were given ovulation-inducing drugs for other purposes for example, super ovulation. All patients had normal baseline androgen levels before they began therapy. Women were also required to have bilateral tubal patency established by hysterosalpingography. Male factor was excluded by requiring a minimum motile density (progressive sperm) of $10 \times 10^6/\text{ml}$; cervical factor was excluded by finding at least three sperm per high powered field with progressive motility 10 h or more after intercourse. The women were divided into two study groups: Group 1 consisted of 40 women treated with clomiphene citrate; Group 2 consisted of 40 women treated with human menopausal gonadotropins (hMG).

Each woman was also required to demonstrate at least one mature follicle during her first cycle of therapy with follicle-maturing drugs, in order for androgen levels to be evaluated. A mature follicle was defined as a follicle attaining an 18–24 mm average diameter as demonstrated by sonography, and a serum estradiol level of 200 pg/ml. Serum samples were collected during menses, and at the time of a mature follicle, for evaluation of testosterone, free testosterone, androstenedione and dehydroepiandrosterone sulfate (DHEA-S) levels. Serum estradiol and luteinizing hormone (LH) levels were measured at the time of a mature follicle only.

DHEA-S and free testosterone levels were measured by solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, California), as were testosterone (ICN Biomedicals Inc., Costa Mesa, California) and androstenedione (Diagnostic Systems Laboratories, Webster, Texas). Estradiol was also measured by solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, California).

To evaluate whether the mid-cycle androgen levels were elevated, the following normal female ranges (as established by the kit's manufacturer) were used; testosterone: 20–80 ng/dl; free testosterone: 0.7–3.6 pg/ml; and androstenedione: 10–299 ng/dl. The manufacturer of the DHEA-S kit reported a normal range of 35–430 $\mu\text{g}/\text{dl}$ based

on data from 132 women. Since the upper level of normal is higher than in many published references, its veracity was challenged by performing the assay on 210 female patients with no clinical indications of androgen excess. This work yielded a DHEA-S range of 25–410 $\mu\text{g}/\text{dl}$ and it was this range which was used in the evaluation of DHEA-S values in this study.

The mean and standard deviation of the baseline and mid-cycle androgen levels was calculated for each drug therapy group. The mean and standard deviation for the number of mature follicles attained and the estradiol and LH levels at the time of follicular maturation were also calculated for each drug therapy group.

The relative frequency of elevated mid-cycle androgen levels was compared between the two study groups using χ^2 analysis with a *p* level of 0.05. Pregnancy rates for patients with normal mid-cycle androgen levels were compared to pregnancy rates for patients with elevated mid-cycle androgens using Fisher's exact test with a *p* level of 0.05.

The relationship of mid-cycle estradiol levels to mid-cycle androgen levels and pregnancy rates was evaluated by calculating the ratio of mid-cycle estradiol to mid-cycle androstenedione and comparing the mean value of the ratio between pregnant and non-pregnant women. A two-tailed Student's *t*-test was used.

RESULTS

Five women were dropped from the study because of insufficient sera to perform all assays (two from Group 1 and three from Group 2). The mean baseline and mid-cycle serum levels for the androgens DHEA-S, testosterone, free testosterone and androstenedione, for the two treatment groups are seen in Table 1.

The distribution of mid-cycle androgen levels that were either within the normal range or elevated, for each treatment group, are recorded in Table 2. The number of patients exhibiting elevated androgen levels was the same in both the group taking clomiphene citrate and the group taking hMG. In both groups, no one had an elevated DHEA-S level. Elevated testosterone values were observed in only one of 38 patients treated with clomiphene citrate and two out of 37 patients treated with hMG. Elevated free testosterone levels

Table 1 Baseline and mid-cycle androgen levels for each follicular-maturing drug group. (Data presented as mean \pm standard deviation)

	Group 1 (n = 38) Clomiphene citrate		Group 2 (n = 37) hMG	
	Baseline	Mid-cycle	Baseline	Mid-cycle
DHEA-S ($\mu\text{g}/\text{dl}$)	212.9 \pm 93.3	193.9 \pm 83.4	178.8 \pm 88.3	162.9 \pm 84.1
Testosterone (ng/dl)	37.7 \pm 20.9	46.4 \pm 25.4	31.5 \pm 14.6	39.4 \pm 24.7
Free testosterone (pg/ml)	1.8 \pm 1.0	2.1 \pm 1.0	1.9 \pm 1.2	2.2 \pm 1.3
Androstenedione (ng/dl)	167.0 \pm 59.3	260.1 \pm 98.0	171.2 \pm 60.9	255.8 \pm 98.9

Table 2 Distribution of mid-cycle androgen levels in women treated with follicle-maturing drugs

	Group 1 (n = 38) Clomiphene citrate		Group 2 (n = 37) hMG	
	Within normal range	Elevated	Within normal range	Elevated
DHEA-S ($\mu\text{g}/\text{dl}$)	38	0	37	0
Testosterone (ng/dl)	37	1	35	2
Free testosterone* (pg/ml)	28	2	22	6
Androstenedione (ng/dl)	25	13	25	12

* Not available for all patients

Table 3 Mean mid-cycle estradiol/androstenedione ratios and serum LH levels according to pregnancy outcome in women treated with follicle-maturing drugs (data presented as mean \pm SD). Numbers in parentheses represent sample size

	Group 1 Clomiphene citrate		Group 2 hMG	
	Non-pregnant	Pregnant	Non-pregnant	Pregnant
Estradiol/androstenedione ratio	2.2 \pm 1.4 (32)	1.7 \pm 0.4 (6)	2.4 \pm 1.5 (32)	3.0 \pm 1.9 (5)
LH (mIU/ml)*	31.2 \pm 31.0 (28)	19.0 \pm 13.4 (6)	27.9 \pm 38.0 (28)	33.5 \pm 15.3 (4)

* LH values were not available for all patients

were observed in two patients taking clomiphene citrate and six patients taking hMG. Elevated androstenedione levels were observed in 13 patients taking clomiphene citrate and 12 patients taking hMG. Thus, treatment with follicle-maturing drugs seems to only affect the mid-cycle level of androstenedione in women with normal baseline values.

Overall, there were 11 pregnancies in the 75 cycles (14.7%), six in the clomiphene citrate group, and five in the hMG group. In the clomiphene citrate group, four of the six pregnancies occurred in women with elevated androstenedione values and two of the six in women with normal androstenedione values. The pregnancy rate for patients who had normal mid-cycle androstenedione levels after one cycle of therapy with

clomiphene citrate was 8% (two of 25) compared to 31% (four of 13) for patients with elevated mid-cycle levels ($p < 0.05$, Fisher's exact test). In the hMG group, three of the five pregnancies occurred in women with elevated androstenedione levels and two of the five in women who had normal mid-cycle androstenedione levels. The pregnancy rate for patients with normal androstenedione levels after one cycle of therapy with hMG was 8.0% (two of 25) as compared to 25.0% (three of 12) when androstenedione was elevated ($p < 0.05$, Fisher's exact test). There were two abortions in the clomiphene citrate group and three in the hMG group.

The mean mid-cycle estradiol/androstenedione ratios according to pregnancy outcome in patients treated with clomiphene citrate or human

menopausal gonadotropins are presented in Table 3. There were no statistically significant differences between the mean estradiol/androstenedione ratio for pregnant and non-pregnant women within each treatment group.

The mean mid-cycle LH values obtained at the time of follicular maturation, prior to the injection of human chorionic gonadotropin (hCG) for release, are presented in Table 3. The LH levels at this point varied greatly within the groups.

In the clomiphene citrate group, women attained a mean of 1.7 ± 0.8 mature follicles in their first cycle of treatment. The mean number of mature follicles in women with elevated androstenedione levels was 2.3 ± 1.0 as compared to 1.3 ± 0.5 for women with normal androstenedione levels ($p < 0.05$, *t*-test). In the hMG group, women attained a mean of 2.0 ± 1.0 mature follicles in their first cycle of treatment. Women with elevated androstenedione levels attained a mean of 2.4 ± 1.2 mature follicles as compared to a mean of 1.8 ± 0.9 for women with normal androstenedione levels (non-significant, *t*-test).

DISCUSSION

In a study published in 1976, Lawrence and colleagues² found that women with polycystic ovarian syndrome had significantly higher plasma androgen levels than women with 'simple' amenorrhea, both before treatment and during induction of ovulation. Even in those patients seemingly ovulating, the pregnancy rate in the women with polycystic ovarian syndrome with elevated androgens was only 21% as compared to 75% in those with uncomplicated amenorrhea². They concluded that 'high levels of circulating androgens might be a factor preventing conception in some patients for whom ovulation is apparently successfully induced'². Our data, however, demonstrated no adverse effects on pregnancy rates in the patients with increased androgen levels.

The results can be interpreted to support the hypothesis that the previously demonstrated negative effects of hyperandrogenicity are related more to the intrinsic disease process rather than to an increase in androgen levels. In fact, the improved

pregnancy rate seen in our study in those with elevated androgen levels might merely reflect the recruitment of more functional follicles, as reflected by the greater number of follicles seen on sonography, and by the higher estradiol levels (since no relationship was seen when evaluating estradiol/androstenedione ratios) and thus, better follicular maturation in the group with elevated androgen levels.

However, an alternate hypothesis is also plausible. The results presented herein in women with normal baseline androgen levels found that only androstenedione, a weak androgen, increased above normal limits, whereas total testosterone and free testosterone (which are more potent androgens) did not. High testosterone and free testosterone, but not androstenedione levels, may exert a negative influence on pregnancy rates, and it is possible that these levels may rise to elevated levels in women starting with high baseline levels.

Previously, Schumert and colleagues³ demonstrated significant elevation of testosterone levels in patients with ovarian hyperstimulation syndrome. None of the patients in our study developed this severe disorder. Most of the women with elevated testosterone levels in Schumert's study developed their increases in the luteal phase³. Our study did not address the question as to whether increased androgen production by the corpus luteum results in an adverse effect on pregnancy rates in women with normal baseline androgen levels. We surmise that the women with the highest mid-cycle androstenedione levels (and the highest pregnancy rates) would be the group most likely to have increased luteal phase androgen levels. However, this increase could remain with elevation of androstenedione only, and not testosterone. An interesting future study would be to evaluate high mid-luteal phase testosterone levels, and to determine whether they exert any detrimental effect on subsequent pregnancy rates.

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