Enclomiphene citrate stimulates testosterone production while preventing oligospermia: a randomized phase II clinical trial comparing topical testosterone

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Objective: To determine the effect of enclomiphene citrate in men with secondary hypogonadism.
Design: Phase II clinical trial.
Setting: Community dwelling men making visits to physician offices.
Patient(s): Men with secondary hypogonadism.
Intervention(s): Oral administration of enclomiphene citrate or 1% topical T gel.
Main Outcome Measure(s): Luteinizing hormone, FSH, T, and semen analysis.
Result(s): Treatment with enclomiphene citrate resulted in increased morning serum T, E_2, and LH levels similar to those obtained with a topical T gel in men with secondary hypogonadism. Follicle-stimulating hormone and LH were increased with enclomiphene, and sperm counts were conserved.
Conclusion(s): Enclomiphene citrate reverses the two hallmarks of secondary hypogonadism, namely, low serum total T and low or inappropriately normal LH while preserving sperm production.
Clinical Trial Registration Number: NCT01270841 (ClinicalTrials.gov Identifier NCT01270841).
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Key Words: Testosterone, testosterone replacement therapy, male infertility, semen, hormones

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Men with secondary hypogonadism are characterized by exhibiting low total T and low to low-normal LH and FSH. Clomiphene citrate (CC) has been used for many years to increase LH, FSH, and T in hypogonadal men (1); however, CC is not approved by the Federal Drug Administration for this use. Enclomiphene citrate (Androxal, Repros Therapeutics Inc), the *trans*isomer of CC, may be able to achieve these same effects in more men. Based on both our preclinical and clinical development, we believe that the use of a single isomer with pure estrogen (E) antagonism (enclomiphene citrate) must have improved activity versus a mixture of isomers (CC) with both antagonism and agonism activities. Federal Drug Administration approval is an advantage for the prescribing physician but those men who have had previously had mixed results on CC should respond better as the isomer that would oppose E antagonism has been removed. Compared with enclomiphene citrate, whose half-life is 10.5 hours, the half-life of zuclomiphene, the other isomer in clomid, is 30 days. This study was undertaken to better determine the effects of enclomiphene citrate on reproductive hormones in men. The investigations were made before, during, and after treatment by using one of two doses of enclomiphene citrate in men with secondary

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hypogonadism. A topical T gel, Testim (Auxilium Pharmaceuticals, Inc.), was used as an active comparator.

Common to all exogenous forms of T supplementation are the potential risks of testicular atrophy and infertility (2–5). Exogenous T can normalize serum T levels, but FSH, LH, and intratesticular T levels are suppressed. Although the state of compromised fertility after T treatment is usually reversible, temporarily impaired spermatogenesis is a significant problem for men who are interested in concurrent fertility. In one recent review, the investigators noted that as many as 25% of urologists may be prescribing exogenous T to their patient in the erroneous hope of "curing" their infertility (6).

In an early phase II study in 52 men, we evaluated the potential of oral enclomiphene citrate to increase serum total T levels in men with secondary hypogonadism (Wiehle et al. 2014, submitted). Men who had low total T values at baseline had significant increases in serum total T levels after 2 weeks of oral therapy with enclomiphene citrate. The increase in total T was accompanied by increases in LH and FSH levels. Our more recently published study provided evidence that enclomiphene citrate increases total T in men (n = 11) with secondary hypogonadism who had been using topical T and also preserved or restored sperm counts (7). This effect was associated with increased endogenous levels of LH and FSH.

The present study investigates men with secondary hypogonadism who were treated with one of two oral doses of enclomiphene citrate or a 1% topical T. This study population either had discontinued previous T treatment for at least 6 months or had never been treated.

MATERIALS AND METHODS

Study Design

The study (ZA-203) was a randomized, phase IIB study, double blind for oral dosage, placebo-controlled, parallel, multicenter study, but open label for the control comparator, that enrolled 124 male subjects in four treatment groups starting January 2011 completing in December 2011. Seventy-three subjects completed the study. The principle investigator provided the Institutional Review Board with all requisite material, including a copy of the informed consent. The study was not initiated until the Institutional Review Board provided written approval of the protocol and the informed consent and until approved documents were obtained by the Principle Investigator and copies were received by the Sponsor (Repros). Initial Institutional Review Board approval for this study was approved on December 7, 2010. Appropriate reports on the progress on the study by the Principle Investigator were made to the Institutional Review Board and the Sponsor in accordance with the applicable government regulations and in agreement with the policy established by the Sponsor. At screening (visit 1) morning hormone levels were determined as well as hemoglobin, hematocrit, glycosylated hemoglobin, and prostate-specific antigen. The subject must have had previous patient history or concurrent diagnosis of secondary hypogonadism. Men could qualify immediately to initiate treatment with a value of <250 ng/dL. Men with values >300 ng/dL were immediately excluded, whereas men with an initial morning serum total T level in the 250–300 ng/dL range were instructed to return to requalify. The morning serum T level was determined by two morning serum measurements (i.e., one at a qualifying visit and the second one at the initiation of treatment visit just before dispensing drug). Blood was collected at the site from all subjects at the same time (9:00 AM) in a fasting condition. At the initiation of treatment visit patients were provided with drug or gel and instructed to apply gel or take capsules at the same time each morning for the duration of the study. Patients were provided with drug or placebo in bottles as randomized by the supplier, not the site or the Sponsor. This was performed by the supplier, blinded to subjects, therefore no bias would be introduced with respect to treatment.

Demographics

Study demographics and patient disposition are listed in Table 1. The safety population represents men who were enrolled and contributed data to baseline values. The intent-to-treat population represents men who were enrolled and received at least one dose. The modified intent-to-treat population are those men who were evaluated after 3 months of treatment. Not all of these men were compliant with supplying semen samples. The men who completed all visits including semen analysis were designated the per protocol population.

To qualify for the study men were selected by virtue of having a morning serum T level of <250 ng/dL on two occasions. This combination of hormone status, age, and body mass index (BMI) is typical of men in our previous studies. There did not appear to be a notable loss of subjects in any one group, although there were more losses from the placebo and topical treatment groups. We also reported percent of men that were oligospermic and the number who presented with LH levels <1.4 U/L.

Semen Analysis

Semen was obtained from subjects by masturbation at the clinical site. Semen analysis was performed at the same site within 1 hour of collection.

Serum Hormones

Routine laboratory measurements were performed at a central site by Cetero Research, Miami Garden, Florida. The FSH was assayed with the ADVIA Centaur FSH assay. This laboratory's normal reference range is 1.4–18.1 IU/L for men 20–70 years of age. Estradiol was assayed with the ADVIA Centaur eE2 assay. Reference range for E_2 in the assay was 0–54 pg/mL. The dihydrotestosterone (DHT) (reference range, 30–85 ng/dL), sex hormone-binding globulin (SHBG) (reference range, 13–71 nmol/L), LH (reference range, 1.7–8.6 MIU/mL), FSH (reference range, 1.5–12.4 MIU/mL), and PRL (reference range, 4.1–18.4 ng/mL) levels were determined using an immunoassay. The total T concentrations in pharmacokinetics subjects (n = 5 for each group) were determined using a validated liquid chromatography/mass spectrometry/mass spectrometry method, with a linear calibration range of 10–5,000 ng/dL and intra-assay and interassay coefficient of variation (CV) of ≤12.8%.

TABLE 1

Study demographics and subject disposition.

	Treatment group (mean ± SD or N [%])			
	Androxal			
Characteristic	**12.5 mg (n = 27)**	**25 mg (n = 33)**	**Topical (n = 33)**	**Placebo (n = 28)**
Age (y)	49.7 ± 11.58	49.2 ± 10.94	52 ± 10.58	51.6 ± 11.7
Subjects oligospermic	5	1	6	2
Subjects LH <1.4 U/L	2	2	1	2
Ethnicity				
White	18 (66.7)	20 (60.6)	23 (69.7)	17 (60.7)
Asian	2 (7.4)	6 (18.2)	6 (18.2)	4 (14.3)
Black or African American	5 (18.5)	6 (18.2)	4 (12.1)	7 (28.0)
Native Hawaiian/Pacific Islander	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)
American Indian/Alaskan Native	1 (3.7)	1 (3.0)	0 (0.0)	0 (0.0)
Height (cm)	178.7 ± 7.53	176.5 ± 8.29	176.5 ± 6.7	175 ± 7.01
Weight (kg)	104.5 ± 18.96	99.3 ± 19.55	103.2 ± 19.38	95.1 ± 17.48
BMI (kg/m^2)	32.6 ± 5.17	31.7 ± 4.9	33.1 ± 5.87	30.9 ± 4.17

	Androxal				
Disposition	**12.5 mg**	**25 mg**	**Topical**	**Placebo**	**Total**
No. of patients randomized	29	33	33	29	124
No. of patients treated at least once	27	33	33	28	121
No. of patients at 3 months of treatment	25	32	30	26	113
No. of patients completed study	18 (62.1%)	20 (60.6%)	19 (57.6%)	16 (55.2%)	73
No. of patients prematurely discontinued from the study	11 (40.7%)	13 (39.4%)	14 (42.4%)	13 (46.4%)	51
Reasons for premature discontinuation from the study					
Adverse events[a]	0	3	1	0	4
Subject withdrawal	2	3	2	3	10
Lost to follow-up	4	2	1	3	10
Patient decision	0	0	1	0	1
Not compliant to protocol	0	5	4	6	15
Other	5	0	5	1	11

Note: BMI = body mass index.
[a] These events were either mild or moderate in intensity, considered possibly related to study treatment and were not serious.

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Statistical Analysis and Sample Size Determination

The primary efficacy variable was the change from baseline for morning T at month 3. An overall comparison among the four treatment groups were performed using one-way analysis of variance (ANOVA). If the overall treatment group comparison was statistically significant, then the pair-wise comparisons were made. No adjustment for multiple testing was used. Because the order of testing was prespecified, no adjustment for multiple testing was required.

The secondary efficacy variables included changes from baseline to month 3 FSH and LH levels. For each of these variables the treatment groups were compared using ANOVA. If the overall treatment group comparison is statistically significant, then the pair-wise comparisons were made.

Safety and tolerability were assessed by evaluating physical and visual acuity examinations, slit lamp eye examinations, clinical laboratory tests, and reported adverse events, as well as changes in values from baseline of semen volume, sperm concentration, total count, morphology, and motility at month 3 comparing Androxal 12.5 and 25 mg with placebo and Testim. These data were summarized for each treatment group separately. Summaries for quantitative variables included the sample size, mean, median, standard deviation, minimum, and maximum. Summaries for categorical variables included the number and percent of patients for each outcome. No formal hypothesis testing was performed to compare the treatment groups.

The primary efficacy variable was the change from baseline to month 3 for morning T. A previous study suggested the SD for the change from baseline to month 3 to be approximately 235 ng/mL. That study also suggested the difference between Androxal and placebo was at least 170 ng/mL. If these assumptions were to be made, along with 80% power and a two-sided significance level of 0.05, it was determined that 30 patients per treatment were needed. As a result, a total of 120 patients were randomized among the four treatment groups.

RESULTS

T, DHT, SHBG, and E_2

A primary end point for the study was the change in morning total T level from baseline to the end of the 3-month dosing period (Table 2). In this analysis only individuals who had an assessment after the baseline visit are included. All three active treatment groups exhibited significant changes in total T level from baseline compared with placebo. There was no statistical difference between the active arms and treatment

TABLE 2

Hormonal changes due to treatment.

Treatment group (subjects)	Mean morning TT (ng/dL, SD) Baseline (visit 2)	Mean morning TT (ng/dL, SD) Month 3 (visit 4)	Change TT from visit 2 to visit 4	FSH (mIU/mL, SD) Baseline	FSH (mIU/mL, SD) Month 3	Change in FSH (SD)	LH (mIU/mL, SD) Baseline	LH (mIU/mL, SD) Month 3	Change in LH (SD)
Androxal 12.5 mg (n = 25)	217.2 (58.8) 202[a]	471.9 (184.6) 461[a]	258.5 (201.5) 227	6.4 (4.2)	11.5 (8.7)	5.1 (6.2)	4.4 (1.8)	8.9 (4.4)	4.8 (4.7)
Androxal 25 mg (n = 32)	209.8 (55.4) 201[a]	405.8 (162.8) 440[a]	197.3 (162.6) 193[a]	9.4 (10.9)	14.9 (10.4)	7.4 (6.5)	5.3 (4.0)	11.7 (8.1)	6.9 (7.7)
Topical T (n = 30)	210.0 (54.0) 201.5[a]	462.6 (289.0) 393[a]	253.7 (293.3) 191.5[a]	6.0 (2.9)	2.4 (2.8)	−4.4 (2.9)	3.9 (1.8)	1.4 (1.7)	−2.4 (2.4)
Placebo (n = 26)	213.7 (74.9) 220[a]	198.5 (72.6) 203[a]	−16.9 (47.5) 21[a]	6.1 (4.8)	5.4 (3.4)	−0.2 (0.7)	3.9 (2.6)	3.7 (2.5)	−0.1 (1.0)

	Before treatment		6 weeks		3 months		After treatment	
Treatment group	**DHT (ng/dL)**	**SD**	**DHT (ng/dL)**	**SD**	**DHT (ng/dL)**	**SD**	**DHT (ng/dL)**	**SD**
Androxal 12.5 mg	15	4.6	22.7[b,c]	9.6	20.4[c]	9.1	13.7	4.3
Androxal 25 mg	15.3	7.2	22.2[b,c]	9.8	23.2[b,c]	20.2	15.8	5.4
Topical T	14.7	5.1	59.2[b,d]	36.3	51.6[b,d]	37.7	19.6	22.7
Placebo	14.4	4.5	17.7	14.9	15.8	7.8	20.1	27.9
	E_2 (pg/mL)	**SD**	**E_2 (pg/mL)**	**SD**	**E_2 (pg/mL)**	**SD**	**E_2 (pg/mL)**	**SD**
Androxal 12.5 mg	20.8	12.4	52.1[b,d]	35.3	56.7[b,d]	31.5	39.1[b]	24.2
Androxal 25 mg	24.7	15.9	48.3[b,d]	28.3	46.3[b,d]	30.7	40.2[b]	27.4
Topical T	26.3	21.8	44.2[b,d]	27.1	37.9[b,d]	21.7	29	16.6
Placebo	22.3	15	26.5	20.3	23.9	17.9	33.3	26.1
	SHBG (nmol/L)	**SD**	**SHBG (nmol/L)**	**SD**	**SHBG (nmol/L)**	**SD**	**SHBG (nmol/L)**	**SD**
Androxal 12.5 mg	24.7	12.9	28.6	14.8	29.3	15.2	24.9	15.2
Androxal 25 mg	26.4	12.1	32	14.5	29.5	12.2	28.7	12.3
Topical T	26.1	10.5	24.6	10.3	25.2	9.8	27.9	16.7
Placebo	27.7	15.5	29.3	16.6	31.4	19.2	26.8	11

Note: DHT = dihydroxytestosterone; SHBG = sex hormone-binding globulin; TT = total T.
[a] Medians.
[b] Different vs. before treatment (each $P < .05$).
[c] Different vs. topical T (each $P < .05$).
[d] Different vs. placebo (each $P < .05$).

Wiehle. Testosterone stimulation by enclomiphene. Fertil Steril 2014.

with topical T when compared with placebo regardless of whether medians or means are considered. Although the topical T treatment exhibited a higher mean morning total T level at 3 months, the median of men receiving topical T was lower than either of the enclomiphene citrate arms, although not statistically significant.

The effects on secondary end points, that is serum DHT, E_2, and SHBG levels, are shown in Table 2. There were no differences among the four treatment groups in terms of DHT, E_2, or SHBG levels before the treatment was started. The mean levels of DHT were increased by both doses of enclomiphene citrate and topical T treatment further increased the level. Results were seen as early as after 6 weeks and were unchanged through 3 months of dosing. The level of DHT provided by topical T was both higher than that seen by enclomiphene treatments and more variable as seen by the SD values. All serum values returned to baseline at the follow-up visit, which was 1 month after the last dose. The levels of serum E_2 showed increases in all three active treatment arms. Increases in serum E_2 were significant versus placebo at 6 weeks and at 3 months. After 1 month of discontinuation of enclomiphene citrate, serum E_2 values remained intermediate between the highest levels seen during treatment and baseline values. There were no significant changes in mean SHBG during the study for any group.

LH and FSH

Effects of treatment gonadotropins are shown in Table 2. All groups were within normal range before treatment. There was an increase in the levels of LH and FSH with treatment of either dose of enclomiphene citrate. The effects were seen after 6 weeks and persisted until the end of the treatment phase. The placebo group showed little change, but the topical T group was associated with a decrease in the two hormone levels. The levels of LH for the enclomiphene citrate in the 25-mg and 12.5-mg arms were 6.7 ± 5.4 and 4.8 ± 2.8 mIU/mL, 1 month after dosing. We identified a persistence of FSH levels in the enclomiphene citrate 25-mg and 12.5-mg arms (9.5 ± 5.4 and 6.6 ± 4.4 mIU/mL, respectively) at 1 month after dosing cessation. Elevated LH values failed to persist 1 month after dosing cessation. Levels of gonadotropins found 1 month after treatment were similar to those found before treatment. For the purposes of the trial, we considered that a normal level of FSH to be 2–12 mIU/mL and LH to be 2–10 mIU/mL. We considered levels of 1.5 mIU/mL and 1.4 mIU/mL of FSH and LH, respectively, to be the lower limits of normal. Treatment with topical T gel suppressed gonadotropins to less than normal range. These comparisons are given in Table 3.

Sperm Concentration

Sperm concentrations was a coprimary end point for this study. Of the 121 men in the population analyzed, 73 men completed the study and provided both baseline and at least one end of study semen sample. There were 20, 23, 14 and 23 men in this group in the 12.5 and 25 mg enclomiphene citrate, placebo, and topical T arms, respectively. Figure 1 shows a lower sperm concentration after 3 months of treatment for the topical T group. Mean and median values are shown in Figure 1. There was a clear difference among the median values of sperm concentrations dependent on treatment ($P=.003$, Kruskal-Wallis test). The topical T group demonstrate lower values for sperm concentration compared with the 12.5 mg and 25 mg enclomiphene citrate groups ($P=.008$ and $P=.0007$, Mann-Whitney U, Wilcoxon test) as well as compared with the placebo group ($P=.007$, Mann-Whitney U, Wilcoxon test). The two enclomiphene citrate treatment groups did not differ from each other ($P=.58$, Mann-Whitney U, Wilcoxon test) or from placebo ($P=.95$ and $P=.78$ for the 12.5-mg and 25-mg groups, respectively, by the Mann-Whitney U, Wilcoxon test). Using the lower limits of normal for sperm concentrations of 15 million/mL (8), Table 3 shows the shift from baseline to the end of study for the men in the four study arms (n = 67) who had at least two assessments at the end of 3 months.

In the course of the study, many men became oligospermic or azoospermic when treated with topical T. Using the figure of 15 million/mL to differentiate the oligospermic from normospermic subjects, we determined that 54% of men who were treated with topical T became oligospermic. In comparison, the proportion of oligospermic men with enclomiphene citrate at any time after treatment was 15 of 103 (14.6%), a lower proportion ($P=.0001$, Fisher's two-tailed exact test). The proportion of oligospermic men on topical treatment was also higher than those on placebo ($P=.0001$, Fisher's two-tailed exact test), whereas the proportion of men made oligospermic after either placebo or enclomiphene citrate treatments did not differ from each other ($P=.60$, Fisher's two-tailed exact test).

TABLE 3

Gonadotropin and sperm concentration changes.

Treatment group	Men < LLN for FSH at month 3	Men < LLN for LH at month 3	Men with sperm concentration < 15 × 10^6/mL at baseline (single assessment)	Men with mean sperm concentrations < 15 × 10^6/mL at the end of dosing (at least 2 assessments)	Difference from topical T at end of dosing (χ^2)
12.5 mg	1 of 26	1 of 26	3 of 16	2 of 16	0.013
25 mg	0 of 30	0 of 30	1 of 19	0 of 19	0.0002
Topical T	13 of 31	17 of 31	3 of 19	10 of 19	–
Placebo	2 of 24	4 of 24	1 of 13	2 of 13	0.033

Note: LLN = lower limits of normal.

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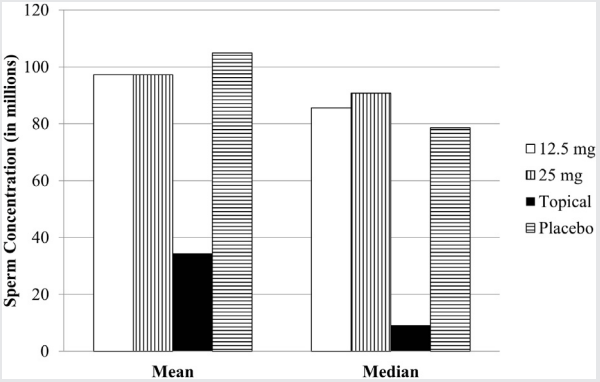
FIGURE 1



Effect of treatment on sperm concentration. Men displaying a morning T value <250 ng/dL were enrolled in the study. Treatment was enclomiphene citrate at 12.5 mg (*horizontal lines*), 25 mg (*vertical lines*), topical T (*solid black bars*), or a placebo (*solid gray bars*). Daily treatment continued for 3 months. Men were asked to provide two semen samples for analysis 3–5 days apart at the conclusion of the study. Data shown are the average for all individuals who provided two or more semen samples.

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The number of subjects who were initially normospermic but became oligospermic was informative. That number was 10, 3, 2, and 1 for the topical T, placebo, 25-mg enclomiphene citrate, and 12.5-mg enclomiphene citrate groups, respectively.

Total Sperm Count

The sperm counts per ejaculate are dependent on sperm concentration and ejaculate volume. The use of topical T was associated with a loss of median total sperm count in the ejaculate. After 3 months of treatment (visit 4), there were clear differences among the four groups ($P=.013$, Kruskal-Wallis test) and the 12.5- and 25-mg enclomiphene citrate groups were not different from placebo ($P=.45$ and $P=.77$, respectively, Kruskal-Wallis test) they were higher and different from the topical T group ($P=.004$ and $P=.012$, respectively, Kruskal-Wallis test). The topical T group was numerically lower in median total sperm counts than the placebo group but that difference failed to reach statistical significance ($P=.056$, Kruskal-Wallis test). The end of study period is approximately 1 month after cessation of treatment. At this sampling the 12.5-mg enclomiphene citrate group was significantly higher than the topical T group ($P=.036$) and the 25-mg enclomiphene citrate group was higher but missed significance ($P=.055$, Kruskal-Wallis test). Only the topical T groups was significantly lower than the placebo group ($P=.026$, Kruskal-Wallis test). Comparing the baseline values of total count between groups after 3 months placebo showed no difference ($P=.92$, Mann-Whitney *U*, Wilcoxon test); the 12.5-mg dose showed no difference ($P=.23$, Mann-Whitney *U*, Wilcoxon test); the 25-mg dose showed no difference ($P=.87$, Mann-Whitney *U*, Wilcoxon test), but the topical treatment group was associated with statistically lower total sperm counts ($P=.0034$, Mann-Whitney *U*, Wilcoxon test). In addition, the same pattern was observed 7 days after the last treatment, but there were no differences in total sperm count at the end of study compared with baseline.

Effects on Other Sperm Parameters

All mean semen volumes were at or more than lower reference limit for the normal range of 1.5 mL (1.4–1.7 mL, 95% confidence interval). In general, effects on progressive motility, total motility, and morphology stayed at more than the normal limit of 40% motile, 32% progressive motile, and 4% normal morphology, except for the topical T group that decreased to less than the 40% level for the total and 32% level for the progressive motility. The progressive motility for all the sperm samples were not different among the groups before drug treatment ($P=.86$, Kruskal-Wallis). The enclomiphene citrate and placebo groups were not different during the course of the trial and remained at more than the 40% lower limit of normal motility. The topical treatment group was less than the 40% motility cutoff value after the 3-month assessment and at 7 days after the 3-month assessment. This difference was significant when comparing the mean values before treatment and the 3-month values ($P=.005$, Kruskal-Wallis). The mean percentage of progressive motility and mean total motility was the same for sperm samples evaluated before and 1 month after treatment. Mean total motility was not different among the four treatment groups before treatment ($P=.88$, ANOVA) and also did not differ among each other after treatment had been halted for 1 month ($P=.66$, ANOVA). There were no differences among the enclomiphene citrate and placebo treatment groups during the course of the study. However, the topical treatment groups demonstrated the lowest mean total motility of any groups at the 3-month + 7-day assessment period ($P=.027$, Kruskal-Wallis). That lower mean value was significant compared with the before treatment mean ($P=.0095$, *t* test). The mean values for morphology were relatively constant during the course of the study and did not approach the lower limit of 4%.

DISCUSSION

Enclomiphene citrate increased total T levels accompanied by increased LH and FSH levels. There were also increases in DHT, particularly with topical treatment and in E_2 with all three active treatments.

We had previously shown that the effects on serum total T were more pronounced for the 25-mg dose than for the 12.5-mg dose (7, 9). In the present study two doses of enclomiphene citrate resulted in morning T levels that were comparable. These three study populations were men with secondary hypogonadism who were similar in age and other demographics. However, the men in the current study were more severely hypogonadal due to the entry criterion of a morning total T level of <250 ng/dL as opposed to the <300 ng/dL standard used previously. The median values for all four groups were <221 ng/dL. We show here that the ability to increase total T levels in men into the normal range was comparable for the three treatment groups.

Estradiol showed increases in all three active treatment arms of the study. The increases in serum E_2 were significant versus placebo. It must be remembered that treatment with

enclomiphene has two effects with regard to E_2. First, there was an increase in E_2 secondary to the increase in total T with a time course that would mirror total T (10, 11). Second, enclomiphene has selective E receptor modulators-like properties most consistent with those of an antagonist (10). Thus, E action evoked by enclomiphene citrate will always be effectively less than that measured as serum E_2 because some E antagonist activity is in the serum as well. In addition, many selective E receptor modulators can have tissue-to-tissue-specific actions. One month after treatment discontinuation, the levels of serum E_2 remained intermediate between those seen at baseline and at 6 months. This persistence of a hormone after stimulation by enclomiphene citrate has been seen before for total T, LH, and FSH, but not for E_2 (12). We term these phenomenon legacy effects. The persistence of E_2 in the absence of T at 1 month is a unique finding.

Total T, DHT, and E_2 are all bound by SHBG. Circulating SHBG bound by hormones represents a kind of buffer of steroid hormone in the body. There were concomitant increases in serum DHT and E_2 without an increase in SHBG. The effects on SHBG are likely to be due to the balance between total T, E_2, and enclomiphene (E antagonist). Enclomiphene reaches a peak within 2 hours of ingestion, whereas the levels of serum T provoked by enclomiphene follow a daily pattern, one more similar to stimulation of testicular production of total T through the enhancement of LH (9). Presumably the levels of the T metabolites (DHT and E_2) would mirror that of serum total T. We have no further way to balance these effects, but we do note the high levels of DHT in the men taking the topical treatment.

The effects of enclomiphene citrate are best characterized as enhancing the levels of gonadotropins LH and FSH. These data are in agreement with the effects seen in previous studies (7, 9). These changes are in contrast with those observed with an exogenous androgen in which similar serum levels of total T were associated with significant suppression of LH and FSH. We have previously shown that legacy effects of enclomiphene citrate on T, LH, and FSH levels that persisted at least 1 week after discontinuation in this study (9). We see a persistence of E_2 for at least 1 month after the cessation of dosing, although we cannot predict the length of this effect. We infer that the persistence of total T, LH, and FSH is at least 1 week, but not longer than 4 weeks. In the case of topical T, the suppression of gonadotropins is relieved after 1 month. It is interesting to speculate that the legacy effect is due to restoration of normal pulsatile LH activity.

Adverse events leading to discontinuation off study included mild hives in one subject, which was considered probably not related to study drug, inability to climax and loss of sensation during intercourse, experienced by one subject, which was considered possibly related to study drug, and mild nausea and mild dry heaving in one subject, which was considered possibly related to study drug. All subjects experiencing adverse events were in the 25-mg group and were described as mild to moderate.

Supernormal levels of total T may be achieved with hormone replacement with both injectable and topical agents (12). The recognition that exogenous T can have suppressive effects on spermatogenesis has been noted by the manufacturers of approved products as well as the Endocrine Society. The Clinical Practice Guidelines from the Endocrine Society (12) state that "reduced sperm production and infertility" are potential adverse effects of T replacement in adult men with androgen deficiency syndromes. Product package insert reports a possible reduction in spermatogenesis (5). Recent reviews of this subject has re-enforced this understanding (4, 6).

In a previous study we reported that men who had been on exogenous T had low sperm counts, but when switched to enclomiphene citrate demonstrated sperm counts in the normal range (7). In the present study, we enrolled men with secondary hypogonadism who reported themselves naïve to topical T. We showed that men in the enclomiphene citrate arms had or continued to have normal sperm parameters. However, men treated in a topical T arm showed declining sperm counts. This study selected for men with more severe secondary hypogonadism as determined by their entry morning total T levels. Most subjects exhibited sperm concentrations at more than the World Health Organization guideline lower limit of normal of 15 million sperm/mL. In this relatively short 3-month study, not only were LH and FSH levels suppressed in the topical T arm but the lowered FSH value apparently translated into lower sperm concentration that decreased to the World Health Organization guideline lower limit in a little more than 50% of subjects. For those men who entered the trial in a normal state, the enclomiphene citrate and placebo arms maintained semen quality at more than the lower limits during the course of the study.

It may be of interest that the variability in sperm number in the topical T group is considerable with some individuals experiencing little loss in sperm whereas others are almost totally lacking. Whether this effect represents individual differences in skin permeability, lack of T response, or compliance with the protocol is not known. On the other hand, men on the topical treatment could recover sperm counts once treatment is discontinued, given that 1 month or less separates the last two semen assessments. It also deserves noting that most men (9 of 12) who were oligospermic at the time of drug initiation, remain so regardless of treatment. This last observation gives no comfort to those men with low sperm counts who wish to regain fertility with enclomiphene, at least within the 3-month duration of this study.

Overall the safety profile of both dose levels of enclomiphene citrate was acceptable. The persistence of T after a drug holiday could take on a practical advantage for men who would take enclomiphene citrate to increase total T levels as they would have some confidence that missing 1 or 2 dosing days would not mean that their total T level would decrease precipitously. Levels of total T, upon missing a few days of topical administration, decreased well below lower limits. Maintenance of stable blood total T levels are required for a clinically relevant effect. The use of enclomiphene may be preferred by those men who wish to have stable serum total T levels. This is in distinction to all other known topical hormone replacement therapies.

The limitations of this study are several. Not all men produced two semen samples for analysis, thus the hormone data are more complete that the semen analysis data. Although men agreed to provide four samples in total of semen for

analysis, in practice some patients were not compliant. All hormone values were determined by immunologic-based assays. We realize that liquid chromatography mass spectrometry assay are replacing older assays; however, immunoassays are more commonly used in clinical practice and this was the case when we initiated the study. On the other hand, all hormone analysis were performed at a central laboratory with consistent internal controls. In the present study semen analyses were performed at the site of collection in a timely manner within 1 hour. This phase II study demonstrated some differences among groups in terms of sperm count. This seems to be random variation and clear statistical differences still could be found, but future phase III studies would benefit from more men enrolled.

In conclusion, enclomiphene citrate reverses low serum total T levels and inappropriately low normal serum LH levels. A topical T replacement agent will increase T equally well but suppress LH and FSH. The elevations of LH, FSH, and total T in men taking enclomiphene citrate accompany the positive effects we have seen on sperm counts, and contrasts with the suppressive effects of exogenous T delivery systems on sperm counts. This phase II clinical trial needs to be repeated in a larger population.

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