

# Oral enclomiphene citrate raises testosterone and preserves sperm counts in obese hypogonadal men, unlike topical testosterone: restoration instead of replacement

Edward D. Kim, Andrew McCullough\* and Jed Kaminetsky†

University of Tennessee Graduate School of Medicine, Knoxville, TN \*Urological Institute of Northeastern, New York, NY, USA, and †University Urology Associates, New York, NY, USA

## Objectives

To determine the effects of daily oral doses of enclomiphene citrate compared with topical testosterone gel treatment on serum total testosterone (TT), luteinising hormone (LH), follicle-stimulating hormone (FSH), and sperm counts in men with secondary hypogonadism.

## Patients and Methods

Two parallel randomised, double-blind, double-dummy, placebo-controlled, multicentre, phase III studies were undertaken to evaluate two doses of enclomiphene citrate vs testosterone gel (AndroGel® 1.62%) on TT, LH, FSH, and sperm counts in overweight men aged 18–60 years with secondary hypogonadism. Men were screened and enrolled in the trials (ZA-304 and ZA-305). All enrolled men had early morning serum TT levels in the low or low normal range ( $\leq 300$  ng/dL;  $\leq 10.4$  nmol/L) and had low or normal LH ( $< 9.4$  IU/L) levels measured on two separate occasions 2–10 days apart. Serum samples were obtained over the course of the study to determine relevant hormone levels at baseline and after 16 weeks of treatment. Men provided semen samples twice to enroll at the beginning and twice at the end of the study.

## Introduction

Although testosterone-replacement therapy (TRT) is widely used in the treatment of hypogonadism there are concerns identified by the Endocrine Society and in the labelling of these products for men wishing to preserve fertility [1,2]. An alternative approach is based on testosterone restoration with selective oestrogen antagonists acting in the pituitary via the hypothalamic–pituitary–gonadal axis. One such drug widely used ‘off-label’ in this indication is clomiphene citrate (Clomid®) [3], which is a mixture of two geometric isomers with different properties. Enclomiphene citrate (enclomid, the

## Results

TT levels increased between baseline and after 16 weeks of treatment in all the treatment groups. FSH and LH levels increased in the enclomiphene citrate groups and decreased in the testosterone gel group at 16 weeks. Enclomiphene citrate maintained sperm concentration in the normal range over the treatment period, while there was a marked reduction in spermatogenesis in the testosterone gel group.

## Conclusions

Enclomiphene citrate consistently increased serum TT, LH and FSH, restoring normal levels of serum TT. Enclomiphene citrate treatment maintained sperm concentrations in the normal range. The effects on TT were also seen with testosterone replacement via testosterone gel but sperm counts were not maintained.

## Keywords

serum testosterone, selective oestrogen receptor modulator, spermatogenesis secondary hypogonadism, topical testosterone

*trans*-isomer of clomiphene, Androxal®) has effects consistent with oestrogen antagonism whereas zuclomid (the *cis*-isomer) often acts as an agonist [4,5]. The clearance of each isomer from the blood differs with zuclomid persisting for much longer [6–9]. Clomiphene citrate is used to raise sperm counts in men previously on exogenous testosterone treatments [10] and in men with prior steroid abuse [11]. Although widely used in women and having been used for many years to increase LH, FSH and total testosterone (TT) in men with idiopathic infertility and/or secondary hypogonadism, Clomid is not approved by the European or Federal Drug Administration (FDA) for use in men.

Male testosterone deficiency is usually categorised clinically as primary or secondary hypogonadism. Of the two, only secondary hypogonadism is the result of deficient gonadotrophin stimulation with consequent impact on testicular function and spermatogenesis, and is often associated with infertility. Spermatogenesis is a complex physiological process contributing to reproductive competency. FSH, LH and testosterone are critical in orchestrating this process. Pulsatile secretion of GnRH from hypothalamic neurones stimulates both LH and FSH secretion from the pituitary gland [12]. LH acts on the Leydig cells in the testes to stimulate secretion of testosterone, while FSH acts on the Sertoli cells in concert with intra-testicular testosterone to promote spermatogenesis. Testosterone, oestradiol, dihydrotestosterone and inhibin B, a protein secreted by the Sertoli cells, provide negative feedback on the hypothalamus and pituitary gland to regulate LH and FSH secretion [13–17].

Hypogonadism in men has been recognised as being reversible [18] and use of exogenous testosterone has been in wide use. Common to all forms of TRT are the potential risks of testicular atrophy and infertility [10]. Exogenous testosterone can normalise TT levels, but FSH, LH and intra-testicular testosterone levels are suppressed. Although the state of sub/infertility after TRT is usually reversible, these effects are critical for men wishing to preserve fertility.

Enclomiphene citrate has been shown in several clinical studies to have a profile that could be beneficial for men with low testosterone who wish to maintain their fertility [9,19–24]. The present studies were undertaken to more fully characterise the clinical profiles of testosterone restoration via oestrogen antagonism (enclomiphene citrate) with TRT (testosterone gel). The results are from two ‘gold standard’ double-blind, double-dummy, placebo-controlled phase III studies designed to meet regulatory guidelines.

## Patients and Methods

Two parallel (ZA-304 and ZA-305) studies were undertaken in overweight men, aged 18–60 years, with secondary hypogonadism. Men were screened with two morning TT measurements to identify individuals for the intent-to-treat population (ITT) by virtue of having a serum TT of <300 ng/dL (<10.4 nmol/L) and a low or inappropriately normal LH level (<9.4 IU/L). These studies were phase III designed to meet regulatory requirements. Both were three-arm studies of double-dummy design for gel and capsule arms with up to 16 weeks of active dosing. In the pooled ZA-304 and ZA-305 studies, 256 men completed the trial in one of four arms. Eighty-six men were in the placebo arm, 85 men were in the Androgel arm, and 85 men received Androxal in two different regimens. Forty-three men received the 12.5 mg dose throughout the study and 42 men were up-titrated to the 25 mg dose of Androxal. The breakdown of the two studies is given in Table 1. The trials required 10 clinic visits with one overnight stay and lasted ≈5 months. At Visit 1, informed consent was taken; an initial screening for TT, LH and FSH was performed, as well as history, vital signs and blood taken for chemistry and haematology. Concomitant medications were assessed. Those meeting initial requirements returned at Visit 2 for concomitant medication assessments, hormones assessment and for the provision of a semen sample. At Visit 3 (baseline) inclusion and exclusion criteria were assessed, as well as vital signs, physical re-examination and concomitant medications assessment. Diet and exercising counselling were performed. At baseline, an eye examination was done, as well as chemistry and haematology, urine analysis, a lipid panel, PSA level, a 12-lead electrocardiogram (ECG), haemoglobin A1c (HbA1c), fasting plasma glucose (FPG), insulin, hormones assessment, and a semen sample taken. Visit 4 (day 0) was the initial day of treatment with study drugs, which were dispensed along with diary cards. At Visits 5 and 6 (weeks 2

**Table 1** Results of the ZA-304 and ZA-305 studies. Effects of treatment on TT levels and sperm concentration.

Study Treatment	ZA-304 ( <i>P</i> values testosterone gel vs enclomiphene citrate)			ZA-305 ( <i>P</i> values testosterone gel vs enclomiphene citrate)		
	Enclomiphene citrate	Testosterone gel	Placebo	Enclomiphene citrate	Testosterone gel	Placebo
N	41	43	45	44	42	41
Mean (SD)						
Age, years (SD)	49.1 (7.4)	47.4 (7.2)	47.2 (9.0)	47.3 (8.8)	45.0 (8.2)	47.5 (8.9)
BMI, kg/m <sup>2</sup>	33.1 (4.4)	34.0 (4.4)	32.6 (4.3)	33.8 (4.6)	33.1 (4.6)	33.5 (4.4)
Baseline TT level, ng/dL	203.3 (52.4)	208.6 (54.0)	200.3 (43.1)	212.9 (48.0)	229.8 (44.0)	206.0 (48.2)
Baseline sperm concentration, million/mL	98.3 (87.2)	78.9 (68.5)	95.3 (90.8)	79.0 (55.2)	75.1 (45.8)	80.5 (62.3)
TT level at 16 weeks, ng/dL	445.8 (186.4)	350.6 (338.1)	236.4 (144.7)	412.9 (130.3)	387.4 (244.8)	214.4 (58.7)
		<i>P</i> < 0.001	<i>P</i> < 0.001		<i>P</i> = 0.037	<i>P</i> < 0.001
% Change in sperm concentration at 16 weeks	11.7 (80.3)	–56.6 (48.2)	4.1 (57.2)	15.2 (55.8)	–32.8 (63.2)	7.6 (89.6)
		<i>P</i> < 0.001	<i>P</i> = 0.548		<i>P</i> < 0.001	<i>P</i> = 0.201
% Sperm concentration <15 10 <sup>6</sup> /mL	4.9	48.8	4.4	2.3	23.8	2.4
		<i>P</i> < 0.001	<i>P</i> = 1.000		<i>P</i> = 0.003	<i>P</i> = 1.000

and 4) the men had their topical gel dose adjusted per manufacturer's instructions, which could be an increase or a decrease. At Visit 6 (week 4) the men who did not achieve a TT of >450 ng/dL (>15.6 nmol/L) on 12.5 mg enclomiphene citrate were up-titrated to 25 mg enclomiphene citrate. Sham up-titrations were done in certain cases. Visit 7 for concomitant medication assessment and study drug pick up for new supply was after visit 6 lab results were available. Visit 8 was at week 10. Visit 9 was the end of treatment at week 16 and was intended as an opportunity for conducting a physical examination with tests. The men returned 1 week ( $7 \pm 3$  days) later for Visit 10. Vital signs were taken at Visits 8, 9 and 10. Semen samples were obtained at Visits 9 and 10 (in parallel to those taken at Visit 2 and 3). Hormone levels were re-assessed at Visits 5, 6, 8, 9 and 10. A serial collection for TT, LH and FSH were done at Visit 9. Assessments of certain blood chemistry values and haematology were performed at Visits 6, 8, 9 and 10 but the full assessment was only at Visit 9. The lipid panel and diet and exercise counselling was repeated at Visit 9. Blood samples for the determination of trough levels of study drug were obtained at Visit 9. Determination of PSA level was done at Visits 6, 8, 9 and 10 to compare with earlier time points. Diary cards were collected at Visits 5 through 9. Levels of HbA1c, FPG and insulin were determined at Visit 9. The combined safety population (SP) was 256 men, which was the same as ITT population (all individuals who had taken at least one dose of study medication). The number of men in the per-protocol (PP) population who completed all portions of the study was 217. Those individuals respond to the drugs relatively quickly [23] and clinically significant changes in TT levels are seen in as few as 14 days of treatment with enclomiphene citrate [24]. Therefore the SP was used for most analyses of adverse events (AEs). The baseline characteristics of the two study populations for both trials are given in Table 1.

Routine laboratory measurements were performed centrally by Cetero Research, Miami Garden, FL, USA. TT (reference range 14–827 ng/dL), oestradiol (reference range 0–146.1 pmol/L), sex hormone-binding globulin (reference range, 27.9–146 nmol/L), FSH (reference range, 1.5–12.4 IU/L) and LH (reference range, 1.7–8.6 IU/L) were assayed with the ADVIA Centaur<sup>®</sup> assay. The ADVIA Centaur assay is a two-site sandwich immunoassay using direct chemiluminometric technology, which uses constant amounts of two antibodies that have specificity for the intact target molecule.

### Semen Analysis

For each man and visit (baseline and end of study [EOS]), the average of assessments was used to determine the total sperm count at a centralised facility (Fertility Solutions, Cleveland, OH, USA). Two assessments were scheduled at

baseline and at the EOS, but all available assessments were used. Men were advised to abstain from ejaculation for at least 3 days. Data were summarised at each visit using descriptive statistics (sample size, mean, median, standard deviation, minimum and maximum). The change from baseline in the semen parameters was compared between treatment groups in a pairwise fashion using an independent two-sample *t*-test or Wilcoxon rank-sum test, as appropriate. Statistical significance of the change from baseline within treatment groups was determined using a paired *t*-test or Wilcoxon signed-rank test, as appropriate. Data from Visits 2 and 3 (baseline visits) and Visits 9 and 10 (end of treatment and follow-up visits) were analysed. Sperm vitality and motility were analysed locally at selected sites only. These data will be presented in future publications, as sperm concentration alone is an insufficient gauge of fertility.

## Results

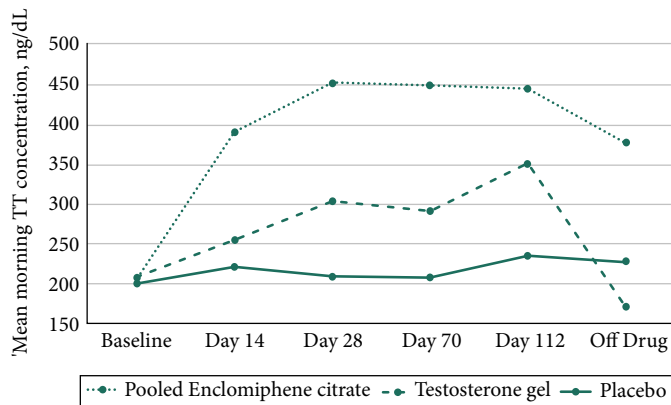
### Baseline Characteristics

The baseline characteristics of ZA-304 and ZA-305 were highly and statistically consistent within studies and between studies for age, body mass index (BMI), sperm concentrations and serum TT. Each individual treatment group, placebo, enclomiphene citrate and testosterone gel, were not different between studies. TT was consistent among the treatment groups in both ZA-304 and ZA-305; however, in ZA-305 the group receiving testosterone gel at baseline, the TT was slightly higher than the corresponding group in ZA-304 ( $P = 0.051$  *t*-test). After treatment (see below) this difference did not manifest any change.

### Baseline Characteristics and Effect of Therapy on TT Levels

There was no difference among the groups at the first or second qualifying visits and values at baseline were indistinguishable [all groups had a mean serum TT of <210 ng/dL and mean range of 200–209 ng/dL]. The time course of the rise in TT over the ZA-304 study is shown in Fig. 1. The TT levels after 2 weeks of treatment for the two highest doses of enclomiphene citrate ('pooled', i.e., 12.5 and 25 mg/day combined) and testosterone gel were higher than for the placebo group. The TT levels increased significantly in all treatment groups between week 2 and baseline ( $P < 0.001$ , Wilcoxon rank-sum test) and between 4 weeks and baseline ( $P < 0.001$ , Wilcoxon rank-sum test). A significant steady state, defined here by TT values of >400 ng/dL, appeared after 4 weeks of treatment with enclomiphene citrate but was not achieved by the testosterone gel group. In the placebo group TT levels remained low throughout the study. There were no significant changes between week 4 and EOS (up to day 112 for the PP population) for any group.

**Fig. 1** Time course of effects on morning mean serum TT levels in men in the ZA-304 study.

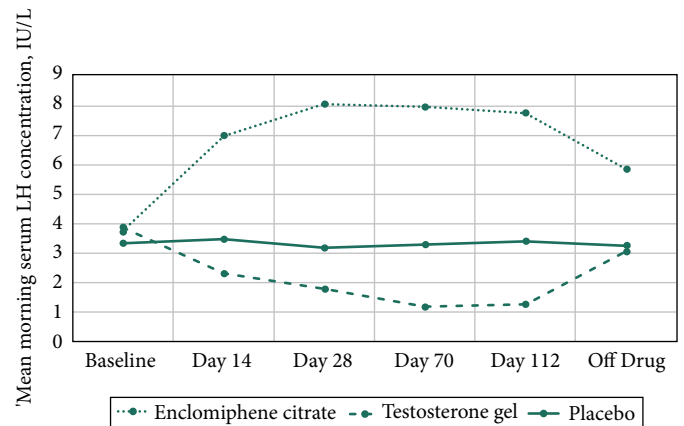


After the cessation of treatment (off-drug point), TT did not return to baseline values for the pooled enclomiphene groups, but remained higher than baseline ( $P < 0.001$ , Wilcoxon rank-sum test) for at least 7 days (Fig. 1). The placebo group TT value did not change vs baseline ( $P = 0.38$ , Wilcoxon rank-sum test) but in the testosterone gel group the TT rapidly decreased such that its final value was less than baseline and similar in value ( $P = 0.07$ , Wilcoxon rank-sum tests). Subtle differences between or among groups was not likely to be due to previous use of gels or injectable, as the proportion of men with a history of previous use was essentially the same between the pooled enclomiphene citrate, testosterone gel and placebo groups (21%, 23% and 21% respectively). Any differences between the pooled enclomiphene citrate and the testosterone gel group in terms of TT, LH, FSH or sperm counts (see below) is also unlikely to be due to the proportion of Type 2 diabetics enrolled, as it was not statistically different ( $P = 0.16$ , Fisher's exact test).

### LH and FSH Secretion

The time course of LH over the study is given in Fig. 2. At baseline the mean LH values were indistinguishable and in the low normal range (mean values 3.3–3.8 IU/L). Values of serum LH increased for the pooled enclomiphene citrate group ( $P < 0.001$ , Wilcoxon rank-sum test) but decreased for the testosterone gel group ( $P < 0.001$ , Wilcoxon rank-sum test) and showed no change in the placebo group ( $P = 0.62$ ,  $t$ -test) during the first 2 weeks of treatment. There was an apparent slight increase between week 2 and week 4 in LH for the pooled enclomiphene citrate group, which was essentially maintained until the EOS or day 112. The mean serum LH values were  $>6$  IU/L after 4 weeks of treatment with the pooled doses of enclomiphene citrate. There were no changes from baseline to the EOS ( $P = 0.98$ ,  $t$ -test) for the placebo group, whereas the testosterone gel group had a clear decrease in serum LH vs baseline ( $P < 0.001$ , paired  $t$ -test).

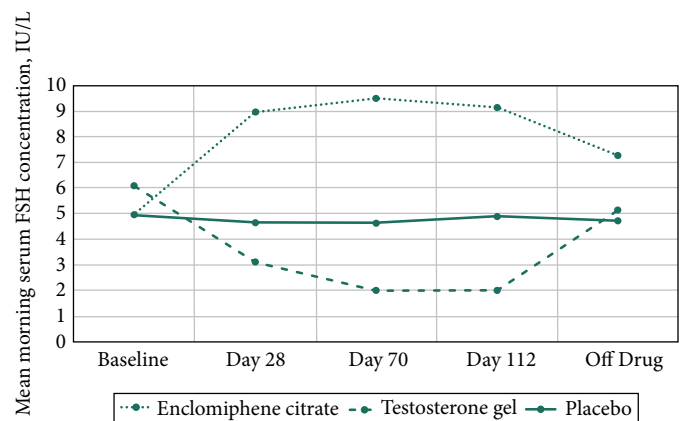
**Fig. 2** Time course of effects on morning mean serum LH levels in men in the ZA-304 study.



After cessation of treatment, the mean LH levels for men receiving enclomiphene citrate were higher than those for men in either the placebo or testosterone gel groups. The mean LH for men on testosterone gel decreased over this week period and it was lower but similar to baseline values ( $P = 0.020$ , Wilcoxon rank-sum test). However, the mean LH levels in men on enclomiphene citrate remained significantly lower than baseline values ( $P = 0.003$ , Wilcoxon rank-sum test).

The time course of FSH over the study is given in Fig. 3. As was the case for LH, levels were similar at the first and second qualifying visits. At baseline the mean FSH values were indistinguishable and in the low-to-normal range (mean values 4.9–6.1 IU/L). Values of serum FSH increased significantly for the pooled enclomiphene citrate group ( $P < 0.001$ , Wilcoxon rank-sum test) but decreased for the testosterone gel group ( $P < 0.001$ , Wilcoxon rank-sum test), and showed no change in the placebo group ( $P = 0.10$ , Wilcoxon rank-sum test) during the first 4 weeks of treatment compared with baseline. There was a slight increase

**Fig. 3** Time course of effects on morning mean serum FSH levels in men in the ZA-304 study.



between Week 4 in FSH for the pooled enclomiphene citrate group, which was essentially maintained until day 112. The mean serum FSH values were  $>9$  IU/L after 4 weeks of treatment with pooled higher doses of enclomiphene citrate. There were no significant changes from week 4 to the EOS for the placebo group whereas the testosterone gel group had a clear decrease in serum FSH that appeared to decrease to a trough by or about day 112. After cessation of treatment, the mean FSH levels for men in the pooled enclomiphene citrate group were higher than those for men on either placebo or testosterone gel, as was seen for serum LH. The mean FSH for men on testosterone gel increased over this time period and it remained low but similar to baseline values (5.2 vs 6.1 IU/L).

The effects seen in ZA-304 were mimicked in ZA-305. There was an increase between baseline and the EOS in TT, LH and FSH for the pooled enclomiphene citrate group, with a slight loss in the 7 days after cessation of treatment. The testosterone gel group had an increase in TT but decreases in LH and FSH between baseline and 16 weeks. The placebo group showed little difference in any of these values. The differences between baseline and the EOS values are given in Figs 2 and 3 for LH and FSH, and in Table 1 for TT and for the comparison of the two studies results.

### Sperm Concentration

The effects of treatment for all groups on sperm counts are given in Table 1 and Fig. 4 for both studies in terms of both absolute numbers and change from baseline. This represents both studies in comparison. There was a slight but not statistically relevant increase in sperm counts in the enclomiphene citrate group in ZA-304 ( $P = 0.91$ , Wilcoxon

rank-sum test) and in ZA-305 ( $P = 0.18$ , Wilcoxon rank-sum test). Values for sperm count in the placebo groups were not different from baselines and were not different from their respective pooled enclomiphene citrate group values. In the testosterone gel-treatment groups, the decrease from baseline was significant for ZA-304 and for ZA-305 (both  $P < 0.001$ , Wilcoxon rank-sum test). There was a statistically significant difference when compared with the respective pooled enclomiphene citrate group in ZA-304 and ZA-305 (both  $P < 0.001$ , Wilcoxon rank-sum test). A fuller description of the semen analysis will be presented elsewhere (G. Fontenot, R. Wiehle, manuscript in preparation).

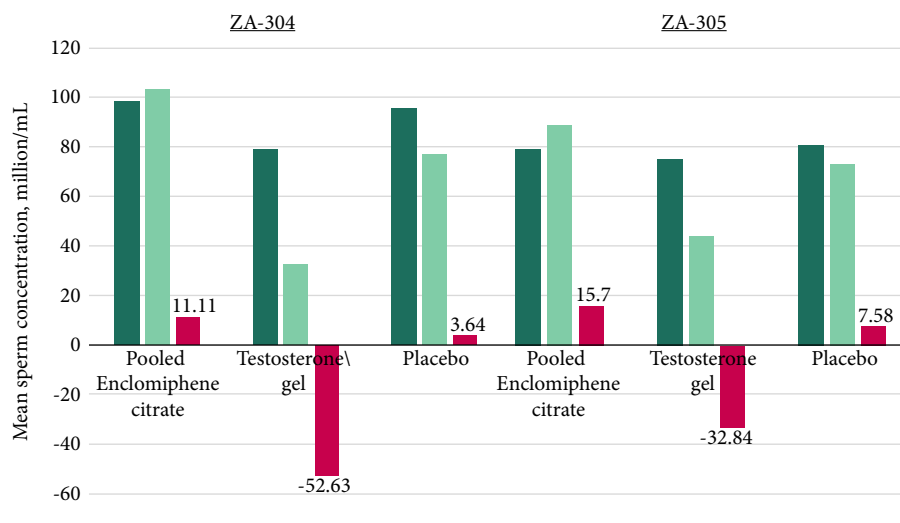
### Composite End-Point Analysis

The use of two primary end-points afforded a composite endpoint in the ITT population for successful treatment. Successes were considered as men achieving sperm concentrations of  $\geq 10 \times 10^6$  and TT in the normal range (300–1 040 ng/dL, 10.4–34.7 nmol/L). Using the data from both ZA-304 and ZA-305, the pooled enclomiphene citrate arms succeeded 63.5%, the testosterone gel arm succeeded 24.7%, and the placebo arm 5.8% of the time. The pooled data for enclomiphene citrate compared with testosterone gel was significantly higher ( $P < 0.001$ , Fisher's exact test) in terms of successes.

### Enclomiphene Citrate Demonstrates Efficacy on Diurnal TT Variation

The hourly variation in TT could be considerable, as we have shown that the use of enclomiphene citrate results in a more normal 24-h profile with a morning rise, a mid-day trough, and an evening increase plus more variability [22,25]. To

**Fig. 4** The mean sperm concentration (million/mL) in ZA-304 vs ZA-305 studies at baseline and 16 weeks, and the percentage change seen from baseline to 16 weeks.





**Table 2** TT<sub>avg</sub> for the serial TT determination up to or after 16 weeks of treatment and distribution using a TT threshold of 300 ng/dL.

	Treatment arms				
	12.5 mg Enclomiphene citrate	25 mg Enclomiphene citrate	Pooled	Testosterone gel	Placebo
<b>ZA-304</b>					
Mean (sd) TT level, ng/dL	446.7 (179.8)	297.7 (106.2)	384.9 (169.2)	346.7 (180.0)	192.7 (49.6)*
CoV, %	40.2	35.7	44.0	51.9	25.7
<300 ng/dL, <i>n</i>	7	8	15	24	43
300–1 040 ng/dL, <i>n</i>	17	9	26	19	2
<b>ZA-305</b>					
Mean (sd)TT level, ng/dL	426.0 (152.7)	338.3 (106.1)	376.2 (134.9)	385.2 (243.9)	201.2 (52.7)*
CoV, %	35.8	31.4	35.9	63.3	26.2
<300 ng/dL, <i>n</i>	4	10	14	19	38
300–1 040 ng/dL, <i>n</i>	15	15	30	22	3

\*Compared to pooled  $P < 0.001$ .

attempt to compare the different potential TT values despite the different diurnal rhythms seen with each treatment, we measured TT over 24 h after 16 weeks of dosing in both studies. The results are given in Table 2. In general, the data for enclomiphene citrate groups were similar to the testosterone gel group in terms of raising the average serum testosterone level over a 24-h sampling period at week 16 (TT<sub>avg</sub>), and both the oral and topical treatment arms were superior to placebo. There was more variability in the testosterone gel arm as seen by the coefficient of variation (CoV) values for the topical treatment in both trials. This has been reported previously [22]. When we compared the ability to raise TT<sub>avg</sub> into the normal range (300–1 040 ng/dL, 10.4–34.7 nmol/L) as opposed to those <300 ng/dL (10.41 nmol/L), it was found that both the oral and the topical treatments were better able to show the more favourable profile compared with placebo ( $P < 0.001$ , Fisher's exact test). Combining the pooled data from ZA-304 and ZA-305 showed that the enclomiphene citrate arms achieved the desired profile more effectively than the topical arm, i.e., a split of more individuals in the 300–1 040 ng/dL range than below it ( $P = 0.030$ , Fisher's exact test) after 16 weeks of dosing.

### Safety Analysis

There were 53 (21%) men in the ZA-304 and ZA-305 studies who had AEs considered by the investigators to be possibly, probably, or definitely related to the study drug. None of these were severe and none were serious. There was no difference in the incidence of treatment-related AEs between treatment groups. No AEs occurred more frequently in the enclomiphene citrate-treated men than in the placebo group. There were seven severe AEs in ZA-304: muscle spasms (placebo), arthropod bite and coronary bypass (testosterone gel group), fatal road traffic accident, hypertriglyceridaemia, cerebrovascular accident and anxiety (12.5 enclomiphene citrate group). There were two severe AEs in ZA-305 study: psoriatic arthropathy and depression in the 25 mg enclomiphene citrate arm. All of these severe AEs were

considered to definitely not be related or probably not related to treatment. Two individuals discontinued the study due to high haematocrit or haemoglobin, one in the testosterone gel arm of the ZA-304 study and the other in the enclomiphene citrate 25 mg arm of the ZA-305 study. One man discontinued due to a high PSA level in the enclomiphene citrate 25 mg arm in the ZA-305 study. There were eight other men who discontinued the study in the placebo or testosterone gel arms.

Other than the road traffic death, the only death was in the ZA-304 study. This was a 59-year-old Caucasian with secondary hypogonadism who was treated in a 'blinded' fashion with 12.5 mg enclomiphene citrate for 34 days before an ischaemic stroke. He had a right middle cerebral artery cerebrovascular accident with hyperdensity consistent with an ischaemic middle cerebral artery. He was treated with tissue plasminogen activator, improved but deteriorated with signs of brainstem herniation. It was likely he was compliant in taking the study drug before the stroke, as his TT had increased from 218 ng/dL (7.6 nmol/L) to 478 ng/dL (16.5 nmol/L). There was no significant change in his haemoglobin after hospitalisation. His haematocrit increased mildly from 46.3% at baseline to 48.4% in the hospital. His past medical history provided evidence that high-risk factors for stroke pre-existed. He was diagnosed with type 2 diabetes 13 years before, he was obese (BMI 41.8 kg/m<sup>2</sup>), he had been treated surgically for atrial fibrillation, which was present on screening ECG but was not on medication, he had sleep apnoea, nephrolithiasis, and hyperlipidaemia (low-density lipoprotein of 137 mg/mL). His high number of risk factors and limited exposure made it highly unlikely that the study medication was the cause of his death in the opinion of the investigator.

### Discussion

We had previously performed a phase II study (ZA-203) that investigated enclomiphene citrate treatment in terms of hormones and semen parameters [23]. Those results

predicted the results seen in the present report, although the treatment protocols were different and all semen analysis was performed at a central laboratory for the ZA-304 and ZA-304 studies. These two pivotal studies reported here provide unequivocal evidence that TRT with testosterone gel and testosterone restoration with enclomiphene citrate have different effects on pituitary and testicular function.

Testosterone gel suppresses pituitary function and spermatogenesis, while enclomiphene citrate, a selective oestrogen receptor modulator (SERM), stimulates LH and FSH production and maintains spermatogenesis. Consistent with prior studies, enclomiphene citrate increases TT levels with the maximum being reached at 28 days [21–23]. Additionally, the increases in TT, LH, FSH levels persisted after cessation of treatment. The positive impact of enclomiphene citrate on LH and FSH almost certainly underlies the lack of deleterious effects on testicular function and sperm count.

One of the basic tenets in medicine is to do no harm. As the present study has shown, exogenous TRT can clearly decrease sperm production and potentially impact fertility potential. Prior to the present study, level 1 evidence of this deleterious effect was not available. Rather, newer formulations of TRT carried the same warning on their label without any data regarding the degree of impact [24]. The use of TRT in hypogonadal men of reproductive age has become a common problem in reproductive medicine. We think that the present study shows that enclomiphene can maintain spermatogenesis while restoring TT levels to normal.

Based on these studies, an ideal patient for enclomiphene citrate therapy is a male with non-classical secondary hypogonadism who desires to maintain his fertility potential. Based on its mechanism of action, a SERM would not be expected to have beneficial impact on a man with pathological secondary hypogonadism. As an example, a male with a history of pituitary irradiation or tumour would not be likely to benefit from enclomiphene citrate as his pituitary gland would be unlikely to increase LH and FSH production.

The ZA-304 and ZA-305 studies, which allowed for an up-titration of the dose in men receiving the lower dose, showed higher TT levels with the enclomiphene citrate 12.5 mg dose than the 25 mg dose. Regardless, men on either dose responded. Previously, in parallel arm dosing, we saw a dose-dependent rise in TT with the 25 mg dose. It is possible that there is a sub-group of men who respond less robustly to enclomiphene citrate treatment but who can be recognised by their ability to respond well to a 12.5 mg oral dose per day. These men who have a strong response at the lower dose may have been subject to selection in some way. This was not due to previous use of TRT products or the presence of type 2 diabetes.

In the present investigation, men on testosterone gel responded less well in terms of TT compared with the values provided in the product label. These were double-dummy designs and well controlled trials and these men were allowed to down-titrate in accordance with manufacturer's instruction if they achieved TT values of >750 ng/dL (26.0 nmol/L). This may not reflect the case of hypogonadal men using a gel under physician's care unless the patient's TT was measured repeatedly for the purpose of dose up- or down-titration. At the same time, testosterone gel was more variable in its effects, a phenomenon we have seen before. While speculative but based on endocrine physiology, higher TT levels with testosterone gel therapy could suppress spermatogenesis and pituitary function more than seen in the present study. Supernormal levels of testosterone may be achieved with TRT with both injectables and topicals [26]. A mid-normal range (350–700 ng/dL) is recommended for adult men with androgen deficiency syndromes [2].

Overall the safety profile of both dose levels of enclomiphene citrate was acceptable. There were few AEs attributable to enclomiphene citrate. There were no unexpected findings regarding cardiovascular safety profile. Changes in PSA and haematocrit seen with both enclomiphene citrate and testosterone gel are secondary to increases in TT. The one death in the two studies combined and not caused by a traffic accident was an ischaemic stroke determined to be unlikely to be caused by the drug.

Study limitations need to be acknowledged. First, we have addressed the less robust response on TT to testosterone gel than seen in their pivotal trials. Second, improvement in patient-reported outcomes (PROs) was not addressed in the present study, which focused on TT levels and spermatogenesis. At the core of this issue is the lack of consensus about whether PROs fully capture improvements in symptoms with therapy; efforts are ongoing to identify and craft the ideal questionnaire. Third, the true impact of a medication on male fertility is difficult to assess without actual pregnancy or live birth data. While far from being a true proxy for fertility, the semen analysis has benefit in this regard. However, for logistic reasons, only sperm concentration was analysed, not sperm motility or morphology. Finally, the duration of the study is relatively short-term compared with the length of therapy seen in clinical practice.

In conclusion, this level 1 evidence shows that enclomiphene citrate reverses the two hallmarks of secondary hypogonadism, namely, low serum TT and low LH. There is also an elevation of serum FSH, which probably accounts for the observed retention of sperm counts. The TT elevating activity of enclomiphene citrate treatment persists for at least 1 week after discontinuation, as a result of maintaining LH. A comparator topical TRT agent, in this case testosterone gel,

will raise TT but suppress serum levels of both LH and FSH. Levels of all three hormones decline rapidly after discontinuation to levels that are lower than baseline suggesting a rapid de-escalation or 'bottoming out' effect. As has been noted previously [21], this persistence of the effects of enclomiphene citrate after the last dose would be an advantage over topical agent, in the sense that skipping a dose would not lead to a rapid bottoming-out that would be seen with the topical agent. The elevations of LH, FSH and TT in men taking enclomiphene citrate underpin the positive effects we have seen on sperm counts and are in marked contrast to the suppressive effects of exogenous testosterone delivery systems on sperm counts. This could be an important consideration in the treatment of hypogonadal men wishing to preserve fertility.

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## Conflicts of Interest

Edward D. Kim and Andrew McCullough are consultants to Repros Therapeutics. Jed Kaminetsky has nothing to disclose.

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**Correspondence:** Edward D. Kim, University of Tennessee Graduate School of Medicine, Knoxville, TN, USA.

**e-mail:** ekim@mc.utmck.edu

**Abbreviations:** AE, adverse event; BMI, body mass index; CoV, coefficient of variation; ECG, electrocardiogram; EOS, end of study; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c; ITT, intent-to-treat population; TTavg, average serum testosterone level over a 24-h sampling period at week 16; PP, per-protocol (population); PRO, patient-reported outcome; SERM, selective oestrogen receptor modulator; SP, safety population; TRT, testosterone-replacement therapy; TT, total testosterone.