Original Research

The effect of three-month topical testosterone gel application on semen quality in men with oligozoospermia and low serum testosterone levels

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Summary

Objective: The effects of daily topical application of testosterone gel on semen characteristics in infertile men with oligozoospermia and hypogonadism were assessed. *Methods*: Sixteen infertile men were included in this prospective study. A daily morning application of 25 mg transdermal testosterone (T) gel was used for 3 months. Semen parameters, serum follicular stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E) and T levels, and seminal plasma E and T levels were measured before treatment and once monthly for three months during the therapy. *Results:* The baseline median sperm concentration was 5.5×10^6/mL. Median sperm concentrations at the first, second and third months were 9×10^6, 8×10^6, and 4×10^6 /mL, respectively. The sperm concentrations at the first and second months after starting therapy significantly ($p < 0.001$) increased compared with the baseline. However, sperm volume, pH, motility, viability and morphology were similar to baseline. Serum T significantly ($p < 0.001$) increased, while serum FSH significantly ($p < 0.001$) decreased from baseline. Seminal T and T/E ratios were raised significantly ($p = 0.008$ and $p = 0.004$, respectively) after gel application, whereas the seminal E level was not affected. *Conclusions:* Daily 25 mg T gel applications for oligospermic infertile men with hypogonadism increased the sperm concentration at the first and second months and raised the serum T level to the normal adult male range. Nevertheless, the sperm concentration had a decreasing trend after the third month.

Key words: Testosterone gel; Oligozoospermia; Hypogonadism; Sperm concentration; Seminal testosterone/estradiol ratio; Male infertility.

Introduction

Infertility affects ~9% of couples worldwide [1] and about 12% in the Thai population [2]. Infertility results from various factors, including male ones, which are solely responsible for infertility in 20%-30% of cases and contribute to 50% of cases overall [3]. Oligozoospermia is associated with infertility. The World Health Organization (WHO) defined the lower reference limit for 'normal' sperm concentration and total sperm number to be 15×10^6 spermatozoa per ml (5th centile, 95% confident interval [CI] $12\text{-}16 \times 10^6$) and 39×10^6 spermatozoa per ejaculate (5th centile, 95% CI $33\text{-}46 \times 10^6$), respectively [4]. Among male factors, 10.1% of infertile men have hypogonadism with low serum testosterone (T) levels and 2.3% show primary hypogonadism [5].

Many hormones and other factors, especially FSH and T, are involved in male reproduction. The hypothalamus produces gonadotropin-releasing hormone, which stimulates the anterior pituitary gland to synthesize gonadotropins, including FSH and LH. FSH regulates the survival of spermatogonia [6] and stimulates B-spermatogonia to differentiate via receptors on Sertoli cells; whereas LH stimulates Leydig cells to synthesize T, which is indispensable for the

normal function of Sertoli cells. Sertoli cells are the nursing cells of male gametes. Zhengwei *et al*. observed that 10 germ cells or 1.5 spermatozoa are embedded in each human Sertoli cell [7]. Hormones regulate spermatogenesis through their effects on Sertoli cells. In mice, many Sertoli cell markers and junctional proteins, such as N-cadherin, connexin-43, gelsolin, laminin-γ3, occludin, testin, nectin, zyxin and vinculin, are androgen-dependent [8]. Several of these molecules are associated with establishing the blood-testis barrier, the release of spermatozoa from the seminiferous epithelium and remodeling of germ cell-Sertoli cell junctions [9]. Testosterone and the androgen receptor are involved in two essential processes of spermatogenesis [10]. First, they act in maintaining the attachment of maturing spermatids to Sertoli cells. Second, they are required for the release of mature spermatids/spermatozoa from the Sertoli cells [10]. However, the collaboration between FSH and T is still inconclusive. Overall, FSH and T act in synergy to produce the factors required to maximize the production of spermatozoa. However, FSH inhibits T-mediated binding of germ cells to Sertoli cells [10, 11]. Consequently, altered FSH levels might be linked to changes in sperm attachment.

Hypogonadism is a rare cause of infertility. It is a clinical syndrome with unequivocally and consistently low serum total T and/or free T concentrations [12]. Serum T levels < 320 ng/dL are suggested to be symptomatic of hypogonadism [12]. Both serum and seminal T and dihydrotestosterone concentrations are correlated with overall sperm concentration and motile sperm concentration [13, 14]. Accordingly, androgen replacement therapy for men with hypogonadism aiming to raise the male sex hormone levels to an optimal level might help reverse abnormal semen production.

Topically applied T gel provides an effective method for T replacement in men with hypogonadism. It was reported to be beneficial in keeping serum T levels steadily within the adult male range [15-17], improving sexual function, mood, increasing lean body mass and decreasing fat mass [15-20]. Moreover, it has advantageous effects for bone mineral density with long-term safety [15-20]. However, data on the efficacy of T gel replacement therapy for spermatogenesis are still limited. A study of the influence of such treatment on spermatogenesis in hypogonadal men with normal semen parameters has shown that the application of 50 mg T gel daily could significantly increase serum T concentration and seminal volume after 3 months [21]. Testosterone is essential for spermatogenesis; nevertheless, supraphysiological T levels suppress LH excretion from the anterior pituitary gland, and consequently decrease intratesticular T synthesis and impair spermatogenesis [22-24]. Despite its suppressive effect, low dose T supplementation is supposed to improve sperm maturation [25] via a nonclassical signaling pathway. Spermatogenesis occurs in the seminiferous tubules. It takes at least 64 days for the germ cell lineage to develop from spermatogonia to mature spermatozoa, or 74 days if including the time for spermatogonial renewal [26]. It takes another 12-21 days to transport spermatozoa from the testis to the ejaculatory duct via the epididymis and vas deferens [27]. Therefore, the whole process needs around 3 months to complete. As a result, any alteration in spermatogenesis induced by T therapy will be evident by about 3 months later.

The primary objective of this study was to investigate changes in sperm concentration in oligospermic infertile men with low serum T levels after 3 months of daily low-dose T gel applications. The secondary objective was to study changes in semen volume, motility, sperm morphology and viability, changes in both serum FSH, LH, T, estradiol (E) levels, seminal T and E levels and T/E ratios in serum and seminal plasma.

Materials and Methods

Patient recruitment and testosterone gel applications

Male participants aged 18 years to 50 years were recruited from patients attending to the infertility unit. Patients diagnosed with oligozoospermia (sperm concentration < 15 million/mL) with serum T levels < 3.2 ng/mL were included. The exclusion criteria included oligo-

zoospermia caused by other factors, for example hydrocele, varicocele, hypogonadotropic hypogonadism (FSH < 1.0 IU/L, LH < 1.1 IU/L) [28] and previous surgery of the testes. Patients who were contraindicated for T usage, such as those with prostatic or breast cancers, allergy to the study drug preparation or previous T replacement therapy were also excluded.

Semen analysis was scheduled for baseline investigation. Samples were obtained by masturbation after 3-5 days of sexual abstinence. The samples were ejaculated into sterile plastic containers at the infertility unit and incubated at 37 °C for 30 min to liquefy. Sperm concentration and motility were analyzed manually according to the WHO Manual 2010 [4] for sperm examination. If the results showed oligozoospermia according to WHO criteria, the semen analysis was repeated several months later. When oligozoospermia was confirmed, the patients were included in the study and the seminal fluids were kept frozen at -80 °C for hormonal profile evaluation later. Baseline serum FSH, LH, E and T levels and testosterone/estradiol (T/E) ratio were also evaluated. After the participants gave their informed consent, they were assigned to apply half a sachet (25 mg) of 1% T gel (Androgel®, Besins Healthcare SA, Brussels, Belgium) daily in the morning to their thighs during three-month period. A blood sample (FSH, LH, E and T levels) and semen analysis were obtained about 3 hours post gel application at every visit (monthly for 3 months) [17]. Any adverse effects, sexual function, medication used, and side effects were reviewed. Unused medications were returned and counted to assess adherence.

Hormone measurements

Serum hormones include follicular stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T) and estradiol (E) were measured in central laboratory in Siriraj Hospital using electrochemiluminescence immunoassay (ECLIA) (Roche Cobas e602 Immunology Analyzer, Germany). FSH and LH were measured by sandwich principle, while T and E were measured by competitive principle. The sensitivity of both serum FSH and LH assays was 0.1 mIU/mL and the sensitivity of serum T and E assays were 0.025 ng/mL and 5 pg/mL, respectively. The intra/interassay coefficients of variation of all these serum hormonal assays were < 5%. Serum T divided by serum E was calculated for serum T/E ratio. Serum FSH, LH, T, E levels and T/E ratio were measured at baseline and post gel application once monthly for three months.

For seminal hormones measurement, the semen samples had been collected and semen analyses performed. Each sample was centrifuged at 400 g for 10 minutes. Aliquots of ~200 μL of the supernatant (seminal plasma) were stored at -80 °C. Thawed seminal plasma T and E levels were measured by competitive enzyme-linked immunosorbent assay (ELISA) techniques. Seminal plasma T and E levels and T/E ratio were measured at baseline and post gel application at every visit for 3 months. All the standards and samples were prepared in triplicate before adding the reaction so-

lutions. Human ELISA kits (Abnova, Taipei, Taiwan), the same one as we used in the previous study, were used for measurement of seminal T levels [29]. The intra/inter-assay coefficients of variation were < 10% and the sensitivity of the assay was 0.173 nmol/L (0.05 ng/mL). This assay uses a highly specific rabbit anti-T antibody as described. Briefly, during incubation, T in test samples and horseradish perox-idase (HRP)-labeled T bind competitively to the antibody, which is also bound by a goat anti-rabbit IgG. After wash-ing, the unbound T-HRP conjugate is removed, and 3, 3', 5, 5'-Tetramethylbenzidine reagent is added, resulting in the development of a blue color. Light absorbance was measured at 450 nm wavelength using an ELx 800™ spec-trophotometer (BioTek Instruments, Winooski, VT, USA).

Human ELISA kits (Cayman Chemical, Ann Arbor, MI, USA) were used for measurement of the seminal E levels. The intra/inter-assay coefficient of variation was < 10%, while the sensitivity of the assay was 15 pg/mL. The per-centages of cross-reactivity for other endogenous hormones including T, dehydroepiandrosterone, dehydroepiandros-terone sulfate, progesterone, and estriol were all < 0.5%.

The wells were coated with mouse monoclonal anti-rabbit IgG. The standard and the study samples were pre-pared in triplicate. During incubation, E in the samples and the E–acetylcholinesterase (AChE) conjugate bind compet-itively to the antibody, which is also bound to mouse anti-rabbit IgG. After washing, the unbound AChE conjugate was removed and Ellman's Reagent was added, resulting in the development of a yellow color. Light absorbance was measured at a wavelength of 412 nm using an ELx 800™ spectrophotometer (BioTek Instruments). The median of the results was recorded and analyzed.

Table 1. — *Baseline characteristics of 16 participants.*

Variables	Mean $\pm$ SD
Age (years)	36.69 $\pm$ 5.3
BMI (kg/m^2)	25.90 $\pm$ 2.3
Serum hormone	Median (P$_{25}$, P$_{75}$)
FSH (mIU/mL)	4.75 (3.6, 7.3)
LH (mIU/mL)	3.32 (2.6, 4.5)
Estradiol (pg/mL)	20.0 (12.5, 23.8)
Testosterone (ng/mL)	2.33 (1.5, 2.7)
Testosterone:Estradiol ratio ($\times 10^2$)	1.2 (0.9, 1.5)

BMI = Body Mass Index, P$_{25}$ = 25th percentile, P$_{75}$ = 75th percentile.

Sample size calculation and statistical analysis

In a pilot study of T gel application to five hypogonadal men with oligozoospermia, the mean increase in sperm con-centration at the third month of usage was $23 \pm 30 \times 10^6$/mL. When α was set at 0.05 and β was set at 0.20, we estimated that 16 infertile men would be needed for

Table 2. — *Semen parameters and baseline seminal testosterone (T) and estradiol (E) concentrations and T/E ratio in seminal plasma.*

Variables	Median (min-max)
Abstinence period (days)	4 (3-5)
Semen analysis	Median (P$_{25}$, P$_{75}$)
Volume (mL)	2.5 (1.4, 3.2)
pH	7.5 (7.5, 7.5)
Concentration ($\times 10^6$/mL)	5.5 (3.9, 8.5)
Total sperm count ($\times 10^6$)	14.45 (9.1, 18.5)
Progressive sperm motility (%)	41.0 (31.3, 52.8)
Viability	62.5 (47.5, 67.8)
Normal morphology (%)	9.0 (5.0, 11.8)
WBC ($\times 10^6$/mL)	0.3 (0.1, 0.5)
Testosterone concentration (ng/mL)	0.53 (0.31, 1.12)
Estradiol concentration (pg/mL)	254.3 (150.3, 424.1)
Testosterone: Estradiol ratio	1.75 (1.1, 4.4)

P$_{25}$ = 25th percentile, P$_{75}$ = 75th percentile.

this study. Statistical analysis was performed using the Statistical Programs for Sciences Software Package (SPSS Inc. Version 20.0, Chicago, IL). Descriptive data are pre-sented as the mean $\pm$ standard deviation (SD) or the median (first and third quartile) for quantitative data with and with-out normal distributions, respectively. Friedman's test was used to compare semen parameters among the four different time points (baseline and at 1, 2, and 3 months) due to posi-tive skewness. In case of significant difference, Wilcoxon's signed rank test was performed for pairwise comparisons with Bonferroni's correction. Bivariate correlations were measured using Spearman's log-rank test. All tests were two tailed with *p* values of < 0.05 accepted as significant.

Results

The baseline characteristics of the 16 infertile men are shown in Table 1. Two patients had underlying illnesses: one had essential hypertension and was taking losartan (50 mg) and atenolol (25 mg) once daily; the other had dyslipi-demia that was controlled with lifestyle modification. All of them denied symptoms of hypogonadism except infer-tility. Two patients are current smokers (12.5%). Baseline semen parameters, seminal T and E levels and the T/E ratios are given in Table 2. Seminal T and E levels in all subjects were above the assay sensitivities.

Changes in semen characteristics are displayed in Table 3. The median sperm concentration increased significantly to 9×10^6/mL (*p* = 0.04) and 8×10^6/mL (*p* = 0.02) at the first and second month compare to baseline, respectively. However, at the third month the sperm concentration had rebounded to be similar to baseline. Other characteristics, including volume, pH, motility and viability, and percent-age of normal sperm morphology at each month were not different from baseline. Serum FSH, LH, E, T levels and T/E ratios and seminal E, T levels and T/E ratios are shown

Table 3. — *Semen characteristics before and after testosterone (T) gel therapy.*

Semen analysis	Baseline (n = 16)	1st month (n = 16)	2nd month (n = 16)	3rd month (n = 15)+	p value
Volume (mL)	2.5 (1.4, 3.2)	2.4 (1.4, 3.2)	2.6 (1.5, 3.0)	2.3 (1.5, 3.0)	0.819
pH	7.5 (7.5, 7.5)	7.5 (7.5, 8.0)	7.5 (7.5, 7.5)	7.5 (7.5, 8.0)	0.720
Concentration ($\times 10^6$/mL)	5.5 (3.9, 8.5)	9.0 (4.0, 14.5)*	8.0 (3.0, 13.8)*	4.0 (2.8, 12.0)	0.189
Motility (%)	41.0 (31.3, 52.8)	44.0 (35.3, 52.0)	35.0 (32.3, 49.5)	37.0 (30.8, 42.8)	0.430
Viability (%)	62.5 (47.5, 67.8)	60.0 (51.8, 67.8)	57.5 (48.0, 63.0)	51.5 (42.5, 65.3)	0.395
Morphology (%)	9.0 (5.0, 11.8)	8.0 (5.0, 10.8)	8.0 (7.0, 10.0)	9.5 (7.0, 10.3)	0.280

All data are presented as medians with ranges between the 25th and 75th percentiles (P_{25} and P_{75}).
*Statistically significant difference ($p < 0.05$) compared with baseline.
+One participant was withdrawn due to adverse event (Transient ischemic attack).

Table 4. — *Serum and seminal plasma hormone levels before and after starting testosterone (T) gel therapy.*

	baseline (n = 16)	1st month (n = 16)	2nd month (n = 16)	3rd month (n = 16)	p value
Serum hormone					
FSH (mIU/mL)	4.75 (3.6, 7.3)	3.48 (1.4, 4.7)*	3.25 (1.2, 5.6)*	3.6 (1.4, 5.8)*	< 0.001*
LH (mIU/mL)	3.32 (2.6, 4.5)	2.52 (1.2, 4.1)	2.59 (1.3, 4.3)	3.04 (1.4, 4.6)	0.737
Estradiol (pg/mL)	20.0 (12.5, 23.8)	30.65 (15.3, 35.5)	28.4 (18.7, 47.8)	21.6 (18.2, 30.5)	0.086
Testosterone (ng/mL)	2.33 (1.5, 2.7)	3.22 (2.6, 6.4)*	4.20 (3.2, 5.5)*	3.02 (2.3, 3.95)	< 0.001*
Testosterone: Estradiol ratio ($\times 10^2$)	1.2 (0.9, 1.5)	1.5 (1.1, 2.4)	1.5 (1.3, 2.0)	1.2 (1.0, 1.6)	0.105
Seminal hormone					
Testosterone (ng/mL)	0.53 (0.31, 1.12)	2.34 (0.54, 15.35)	5.03 (1.13, 12.47)*	3.12 (1.21, 15.62)*	0.008*
Estradiol (pg/mL)	254.3 (150.3, 424.1)	292.2 (183.7, 409.4)	237.3 (181.3, 411.7)	253.3 (203.2, 360.9)	0.615
Testosterone: Estradiol ratio	1.75 (1.1, 4.4)	10.2 (1.6, 55)	16.6 (3.2, 64.3)*	12.7 (2.8, 67.9)*	0.004*

All data are presented as medians with ranges between the 25th and 75th percentiles (P_{25} and P_{75}).
*Statistically significant difference compared with baseline.

in Table 4. Serum FSH significantly ($p < 0.001$) decreased after T gel therapy over all three months after starting T gel therapy compare to baseline. Serum T levels raised significantly ($p < 0.001$) in first and second months, while seminal T levels raised significantly ($p = 0.008$) in second and third months compared with the pretreatment data. Serum LH and serum and seminal estradiol (E) levels were similar to the baseline level. The seminal T/E ratios increased significantly ($p = 0.004$) in second and third months compare to baseline.

Some patients got other benefits of T gel application, such as improvements in libido and sleep quality. As for adverse side effects, two patients developed itching, but this resolved spontaneously. Unfortunately, an ischemic stroke developed in one patient with hypertension after 1 month of using T gel. Although he recovered fully in 3 hours, the study was discontinued.

Discussion

The use of androgen treatment in cases of idiopathic male infertility can lead to a direct effect [30] and a rebound effect [31, 32]. The direct effect means that low-dose androgen application improves epididymal sperm maturation directly; while the rebound effect occurs after stopping androgen supplementation. When androgen-induced gonadotropin suppression is ceased, it leads to a transient rebound increase in the secretion of gonadotropins, which in turn stimulates spermatogenesis. Unfortunately, both rationales lack solid supporting evidence in humans. Failure to find a direct effect can be explained by the supraphysiological T level induced by improper supplementation, which in turn induces negative feedback on the gonadotropin releasing system, leading to a suppression of spermatogenesis.

In this study, the sperm concentrations increased significantly at the first and second month compared with baseline. This increase might have resulted from a synergistic action of two mechanisms. First, the increased intratesticular T levels, which were indirectly represented by increased seminal T level and seminal T/E ratios, could have augmented the release of mature spermatozoa from the Sertoli cells by the first month. Testosterone acts on Sertoli cells via three major pathways: the classical pathway and two non-classical pathways [11]. One non-classical pathway induces Ca^{2+} influx into Sertoli cells, the other is the activation of a series of kinases (within 1 min) which are critical to maintain spermatogenesis. The latter one acts via Src tyrosine kinase and extracellular signal-regulated (ERK) kinases to facilitate the adhesion of immature germ cells to Sertoli cells and also through Src to permit the release of

mature spermatozoa [11]. Thus, increased intratesticular T levels probably facilitate this function. Second, the decreased serum FSH concentration during the first month of T supplementation might have reduced its inhibition of T-mediated binding of germ cells to Sertoli cells. FSH exerts its function on Sertoli cells through at least five signaling pathways, involving cAMP-dependent protein kinase A (PKA), mitogen-activated protein kinase (MAPK), calcium, phosphatidylinositol 3-kinase and phospholipase A_2 [33]. Shupe *et al*. found that FSH inhibited T-mediated activation of the ERK and MAPK pathways in Sertoli cells via the PKA-mediated inhibition of Raf kinase and reduced germ cell attachment to Sertoli cells in culture [10]. As a result, the reduced FSH concentration might theoretically have amplified the function of T. However, sperm concentration at the third month after starting T gel use was not different from baseline. This was similar to the study conducted by George *et al*. In that study, 50 mg daily T gel was used in eugonadotropic/hypogonadic men with normal semen parameters. There was normalizing of serum T levels without suppression of sperm concentration or motility [21]. This might have been associated with the significantly decreased FSH level. Reduced serum FSH might inhibit spermatogonial differentiation and maturation, reflected by the diminished sperm concentration 3 months later in our study. In other words, FSH has a dual effect on sperm production. It has an acute effect on the regulation of T-mediated binding of germ cells to Sertoli cells; and a delayed effect that promotes the transition from type A pale spermatogonia to type B spermatogonia during spermatogenesis [34]. The latter might have been reflected in the decreased sperm concentration around 3 months later in our study.

In the presented study, it was found significant increases in seminal T levels along with serum. The finding suggests that the gel application caused higher intratesticular T levels. Physiologically, these levels in men are about 50-100 fold higher than those in serum [34-37]. Intratesticular testosterone accumulates from at least two sources, one from local production by Leydig cells, the other by transportation from lymph spaces via a binding carrier molecule such as albumin [38]. Therefore, intratesticular androgen levels could be raised by lymphatic transportation, and excreted into the semen. Studies in animal models also found the restoration of testicular T via exogenous T administration [39, 40].

Testosterone is one of the most important sex hormones in men. It is responsible not only for sperm maturation, but also influences sexual function, muscle strength, and lipid and bone metabolism. Here we recruited asymptomatic men with low baseline T levels. After the daily use of 25 mg T gel, serum T levels were raised to the normal adult male ranges as seen in previous studies that used 50, 75 and 100 mg T gel applications [20, 21]. Thus, low-dose T gel applications were adequate for hormone replacement in these hypogonadal men. Even this small amount of gel could effectively suppress serum FSH levels. Besides the effects on serum FSH and T levels, we did not find any significant changes in those for serum LH and E, similar to previous study [41].

A balance between estrogens and androgens in seminal plasma is also important for normal male fertility. Luboshitzky *et al*. reported elevated seminal plasma E and decreased T levels in infertile men, which suggested a correlation between the seminal plasma T/E ratio with sperm concentration [42]. In the present study, seminal plasma T was significantly increased after treatment while the E level did not change, leading to increased T/E ratios. These increased ratios might explain the increased sperm concentrations for the first 2 months after T gel application because T/E ratios in the seminal plasma from oligospermic men were significantly lower than in a normospermic group [43]. Moreover, it has been suggested that selective estrogen receptor modulators and aromatase inhibitors can restore the T/E ratio and improve sperm concentrations [44]. Here, we found a slight positive correlation between serum and seminal T/E ratios when analyzed with Spearman's log-rank test. The range of correlation coefficients was 0.15 to 0.40. Both hormones have their specific functions in the testes. Androgens can be converted into estrogens via the cytochrome P450 aromatase in spermatozoa [45], which is involved in the resorption of fluid in the efferent ducts secreted by Sertoli cells under androgen control [46, 47]. Therefore, a local balance between estrogens and androgens plays an important role in maintaining normal spermatogenesis.

Despite hormonal level alterations, semen volume was not changed after T gel application. Since semen is a mixture of testicular spermatozoa, secretion of seminal vesicle, prostate gland and mucous from bulbourethral gland; semen volume can be affected by other factors beyond testosterone, such as BMI and smoking status.

Transdermal T delivery in this study demonstrated long and steady improvements in serum T levels and was generally well tolerated. Only 10%-15% of the preparation is absorbed into the systemic circulation [48]. One serious adverse event that occurred in this study was an acute cerebrovascular incident. The patient was 50 years old and had essential hypertension. Although this seems not to have resulted from the medication, it was discontinued in any case.

Our study is the first clinical trial demonstrating the effects of T gel on hypogonadal men with oligozoospermia. We found positive effects over the first two months, but a negative effect by the third month. We investigated correlations between serum and seminal plasma T and E levels but the results were inconclusive. One limitation of the study was the inherent variations in semen parameters which might have affected the results. The small sample size was probably insufficient to evaluate the potential and long-term effects of the drug fully. Therefore, well-designed randomized controlled trials are needed to demonstrate the real effects of T gel applications on semen quality in hypogonadal men with oligozoospermia.

In conclusion, daily applications of 25 mg T gel improved sperm concentrations in these oligospermic infertile men with hypogonadism after 1 and 2 months of application. Nevertheless, the use of T gel beyond the third month reduced sperm concentrations. Moreover, daily application of 25 mg T gel in these hypogonadal men could raise serum T levels into the normal adult male range. Further studies are needed to clarify the mechanisms and optimal dosage of T required to improve sperm concentrations in eugonadotrophic hypogonadal men with oligozoospermia.

Ethics Approval and Consent to Participate

This clinical trial was conducted at the Infertility unit, Siriraj Hospital, Mahidol University, Thailand after obtaining ethical approval from the relevant Institutional Review Board (approval number: CoA Si014/2016). It was registered with the Thai clinical trials registry (TCTR20161030001) and conformed to the Declaration of Helsinki. All participants provided informed consent after counseling for infertility treatment and being informed about the study protocol.

Acknowledgments

Conflict of Interest

The authors declare no conflict of interest.

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