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# Treatment of idiopathic oligozoospermia with combined human chorionic gonadotropin/human menopausal gonadotrophin: A randomised, double-blinded, placebo-controlled clinical study

Nan Zhao[1] | Xi-Lan Lu[2] | Jun-Tao Li[2] | Jian-Min Zhang[3]

[1]Department of Andrology, People's Hospital of Liaocheng City, Liaocheng City, China

[2]Center for Reproductive Medicine, Jinan Central Hospital Affiliated to Shandong University, Jinan, China

[3]Weifang Nursing Vocational College, Weifang, China

**Correspondence**
Jian-Min Zhang, Weifang Nursing Vocational College, Weifang, China.
Email: jmzwfhl@163.com

## Abstract

To evaluate whether hCG/hMG therapy has beneficial effects on idiopathic oligozoospermia in Chinese infertility population. The patients were randomly divided into the treatment group receiving hCG/hMG for 3 months and the placebo group receiving placebo for 3 months. Semen and biochemical analysis was performed, and DNA fragmentation as well as spermatid concentration was evaluated. Administration of hCG/hMG for 3 months could significantly improve sperm concentration, rate of forward motile spermatozoa, total motile sperm count, the percentage of sperm with normal morphology and the rate of spontaneous pregnancy in medium- and higher-level inhibin B group respectively. Moreover, in medium- and higher-level inhibin B group, sperm DNA fragmentation index and spermatid concentration were significantly declined respectively at the end of treatment. However, there were no significant differences in lower-level inhibin B group before and after treatment in term of seminal parameters, DNA fragmentation and spermatid concentration. HCG/hMG therapy for 3 months has a beneficial effect on a part of male with idiopathic oligozoospermia, and the efficacy of hCG/hMG therapy is associated with the inhibin B level.

KEYWORDS

human chorionic gonadotropin, human menopausal gonadotrophin, inhibin B, oligozoospermia

## 1 | INTRODUCTION

Male factor infertility is involved in approximately half of cases of sterile couple, although its pathogenesis remains largely unknown (Kumar & Singh, 2015). In most cases, the treatment of male infertility relies on assisted reproductive technology (ART), which is relatively expensive and represents a remarkable example of gender inequity (Chansel-Debordeaux, Dandieu, Bechoua, & Jimenez, 2015). In fact, ART for male factor infertility requires hormonal treatment of the fertile women, who sometimes suffers serious side effects (Nardelli, Stafinski, Motan, Klein, & Menon, 2014). Generally, ART does not foresee pharmacological optimisation of the male partner.

The empiric treatment for idiopathic oligozoospermia includes (a) Anti-oestrogens. (b) Gonadotropin-releasing hormone or human menopausal gonadotrophin (hMG). (c) High purity or recombinant follicle-stimulating hormone (FSH). (d) Androgens. Human chorionic gonadotropin (hCG) is a hormone which is produced by the human placenta, and is found in the urine of pregnant women (Moss, Crosnoe, & Kim, 2013; Valenti et al., 2013). HCG is mainly composed of an LH analog which stimulates Leydig cells to produce testosterone. When given in exogenous form, hCG increases circulating testosterone level and intratesticular testosterone level by stimulating Leydig cells (Coviello et al., 2005; La Vignera et al., 2016). FSH is the hormone which is produced by the anterior pituitary gland, activates the proliferation of the Sertoli cells and induces

  

the mitotic activity of the spermatogonia as well as supports cellular differentiation until the round spermatid stage (Efesov, Cayan, & Akbav, 2009). HMG is a source of FSH to improve spermatogenesis. Numerous patients suffering hypogonadotropic hypogonadism have benefit from the administration of hMG in terms of testicular volume, seminal parameters and pregnancy rate (Finkel, Phillips, & Snyder, 1985; Hosseseinifar et al., 2013; Mao et al., 2007; Yang, Zhang, Dong, Xiong, & Li, 2011). However, there were relatively few studies concerning the effects of hCG/hMG on the male with normogonadotrophic oligozoospermia. Schill, Jüngst, Unterburger, & Braun, 1982 reported that combined hMG/hCG therapy in sterile male with idiopathic oligozoospermia was efficient in a part of cases. However, controversy still exists concerning whether gonadotrophin therapy is beneficial in normogonadotrophic oligozoospermic patients (Knuth, Hönigl, Bals-Pratsch, Schleicher, & Nieschlag, 1987; Lunenfeld, Olchovsky, Tadir, & Glezerman, 1979).

Here, we performed a randomised, double-blinded, placebo-controlled clinical study to evaluate whether combined hCG/hMG therapy has a beneficial effect on idiopathic oligozoospermia in Chinese infertility population.

## 2 | MATERIALS AND METHODS

A total of 316 infertile men with idiopathic oligozoospermia whose sperm concentration consistently below 15 million/ml, age range being from 20 years to 48 years (32.2 ± 2.8 years), were recruited from Center for Reproductive Medicine of People's Hospital of Liaocheng City between June 2015 and September 2017. All participants were properly informed about the purpose of the study and given informed written consent. The study was approved by the ethics committee of People's Hospital of Liaocheng City.

Oligozoospermia was demonstrated in at least three semen analyses performed within a period of 6 months. The men met the following inclusion criteria: infertility for at least 1 year; no medical treatment in the previous 6 months; no presence of varicocele; no smoking; no infection of the accessory sex glands; and no identifiable cytogenetic abnormalities.

All of the wives (mean age 29.6 ± 3.1 years, range 23–35 years) received a complete infertility workup to rule out female factors. All partners ovulated regularly detected by ultrasound; no anatomic abnormalities detected by ultrasound; no abnormal fallopian tube anatomy detected by hysterosalpingography.

All the patients were randomly divided into treatment group and placebo group according to simple randomisation method using Excel 2010 software (Microsoft Corporation, Washington, USA) by "RANDBETWEEN (0;1000000)" function. The allocation sequence produced by the statistician was delivered to our pharmacist. The reproduction specialist in this study did not know about the results of allocation table. Patients ($n$ = 158) in treatment group received intramuscular injections of 2000 IU hCG twice a week in combination with 150 IU hMG three times a week for 3 months, while the remaining patients ($n$ = 158) in placebo group received intramuscular injections of physiological saline solution for 3 months.

Recently, Barbotin et al conducted a prospective study to establish the reference range for serum inhibin B by applying the updated Gen II assay. The results demonstrated that in normozoospermic group ($n$ = 377), the 2.5th percentile for inhibin B was 92 pg/ml and the 97.5th percentile was 316 pg/ml (Barbotin et al., 2015). So, in this study, according to the plasma concentration of inhibin B, the patients in treatment group or placebo group were classified into three groups: lower-level group (inhibin B level <92 pg/ml), medium-level group (92 pg/ml < inhibin B level <316 pg/ml) and higher-level group (inhibin B level >316 pg/ml). At baseline, at the first month, the second month and the third month of treatment, blood samples were collected between 9 a.m. and 10 a.m. after an overnight fast. All samples were centrifuged immediately, and serum was stored at −70°C until assayed for FSH, inhibin B and testosterone. Semen analysis was performed, and sperm morphology as well as DNA fragmentation was evaluated at baseline, at first month, second month and third month of treatment.

### 2.1 | Semen analysis

According to the guideline of WHO, 2010, semen was collected into sterilised glass containers by masturbation after a 3–5 day abstinence. After evaluation of liquefaction and measurement of viscosity and volume, motility was measured at room temperature (22–25°C), 1 hr after ejaculation as previously described (Auger, Jouannet, & Eustache, 2016; Ben Khelifa et al., 2014; Jenkins et al., 2016; Rowe, Comhaire, Hargreave, & Mahmoud, 2000; WHO, 2010). Sperm concentration was calculated in an undiluted semen specimen using Makler's Counting Chamber (Makler, 1980; Panidis et al., 2003).

Sperm morphology evaluation was performed from Papanicolaou-stained smears, and the classification of abnormal sperm forms was made according to the guidelines of the WHO (Auger et al., 2016). One hundred spermatozoa were observed from each semen specimen, and the same individual evaluated all smears.

### 2.2 | Boichemical analysis

FSH (IU/L) was measured using the FSH IRMA kits from Biosource Technologies (Vacaville, CA). Circulating inhibin B level was determined using the ultrasensitive dimmer ELISA kit (Woshide Co., Wuhan, China). The assay detects the complete inhibin B molecule, composed of the α and β chain, but not the free α-chain in serum. Serum T concentration (ng/dl) was measured by enzyme-linked immunosorbent assay (ELISA; testosterone enzyme immunoassay test kit, Woshide Co., Wuhan, China). The assays were run in duplicate, resulting in an intra-assay variation of 4.6%. Measurement of 25OHD level was conducted using isotope-dilution liquid chromatography–tandem mass spectrometry with interassay coefficients of variation (CVs) <10%.

### 2.3 | Sperm DNA fragmentation index

The sperm DNA fragmentation index was evaluated by fluorescence microscopy using terminal deoxynucleotidyl Transferase-mediated dUTP Nick End Labeling (TUNEL) assay (Boshide Co., Wuhan, China) according to the instructions of a commercial assay kit (Gandini et al., 2000). One aliquot of semen sample was washed twice with 0.9% NaCl solution. The pellets were suspended in PBS containing 10% glycerol and transferred to Eppendorf snap-cap tubes. The tubes were stored at −80°C. After thawing, the samples were centrifuged for 5 min at 200 g and then incubated with a solution of 0.1% Triton X-100 and 0.1% sodium citrate (Sigma, St Louis, MO, USA; T-8787) for 2 min on ice. After washing, the slides were dried in air. Thirty microlitres of TUNEL mixture (terminal deoxynucleotidyl Transferase and fluorescein-dUTP) was added to each sample. Samples were incubated for 60 min in a humidified dark chamber, washed three times with PBS and then analysed under fluorescence microscope (Leica DMR; Leica, Wetzlar, Germany). At least 500 cells were counted.

### 2.4 | Immunocytochemical staining

Slides from each group ($n$ = 10) were stained with immunoperoxidase method at room temperature. 01% hydrogen peroxide in distilled water was applied for 15 min to quench endogenous peroxidase activity; a serum-free protein block was applied for 15 min; incubation for 30 min with either monoclonal antibody against human intra-acrosomal antigen SP-10 (Woshide Co., Wuhan, China) or against human antigen CD-45 (Woshide Co., Wuhan, China), or with PBS; peroxidase-conjugated immunoglobulins (goat anti-mouse, Boshide; 1:50 in PBS containing 0.5% BSA) for 15 min; 3,3′-diaminobenzidine tetrahydrochloride (Woshide Co., Wuhan, China) dissolved in 15 ml of Tris/HCL buffer containing hydrogen peroxide for 15 min. Finally, the slides were counter-stained, dehydrated, cleared and mounted under DPX (BDH, Poole, UK). The spermatids in the seminal smear were identified by SP-10 immunoreactive area in the cytoplasm or nucleus. Leucocytes were identified by CD-45 immunoreactivity in which the whole cell was dark brown.

### 2.5 | Statistical analysis

All data are expressed as mean ± *SEM*. Two-tailed statistical significance was set at 5%. The normality of distribution was assessed with the Kolmogorov–Smirnov test (K-S test). The data were assessed by analysis of variance (ANOVA) and repeated-measures ANOVA using the SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered to be significant.

## 3 | RESULTS

Clinical examination showed that all of the patients had complete development of the secondary sex characteristics, with a mean right testicular volume of 17.43 ± 1.24 $cm^3$ and mean left testicular volume of 17.36 ± 1.86 $cm^3$ (mean testicular volume 17.39 ± 1.68 $cm^3$). Total testicular volume was assessed by comparison with a standard value on orchidometry. In this study, there were no significant differences in epidemiologic and somatotopic features among all the groups ($p > 0.05$) (Table 1).

The negative impact of obesity on male reproduction is gradually recognised. Obesity is correlated with reductions in sperm concentration and motility, increase in sperm DNA damage and changes in reproductive hormones (Liu & Ding, 2017). In this study, no significant differences were observed in body mass among all the groups ($p > 0.05$) (Table 1).

Most clinicians recognised vitamin D deficiency defined by a threshold set at 25 nmol/L and insufficiency at 50 nmol/L (Thacher & Clarke, 2011). Study has shown vitamin D deficiency impaired male fertility, which suggests a role for vitamin D in male reproduction (Blomberg et al, 2016). In this study, 25OHD levels of all patients were higher than 50 nmol/L (87 ± 5 nmol/L) in the results.

### 3.1 | Semen analysis

The results of this study demonstrated that at the first month and second month of treatment, the sperm concentration, rate of forward motile spermatozoa, total number of motile spermatozoa and the percentage of spermatozoa with normal morphology did not significantly increase compared with that at baseline. Yet, at the end of 3 months of treatment, there was a significant increase in terms of semen parameters.

At baseline, there were no significant differences in seminal parameters among all the groups ($p > 0.05$). At the third month of treatment, the sperm count, rate of forward motile spermatozoa, total number of motile spermatozoa and the percentage of spermatozoa

**TABLE 1** Epidemiologic and anthropometric features of each group

| | Placebo group ($n$ = 158) | | | Treatment group ($n$ = 158) | | | |
|---|---|---|---|---|---|---|---|
| | Lower level ($n$ = 32) | Medium level ($n$ = 108) | Higher level ($n$ = 18) | Lower level ($n$ = 30) | Medium level ($n$ = 112) | Higher level ($n$ = 16) | $p$ |
| Age (years) | 31.96 ± 3.62 | 33.31 ± 4.16 | 32.45 ± 3.81 | 33.93 ± 4.15 | 32.34 ± 3.92 | 31.96 ± 2.96 | 0.634 |
| Right testicle volume (ml) | 16.26 ± 1.84 | 17.29 ± 1.69 | 16.38 ± 1.75 | 17.46 ± 1.62 | 15.98 ± 1.78 | 18.06 ± 1.69 | 0.365 |
| Left testicle volume (ml) | 17.42 ± 1.26 | 16.48 ± 2.62 | 18.03 ± 1.87 | 17.06 ± 2.02 | 17.13 ± 1.53 | 18.26 ± 2.62 | 0.236 |
| Mean testicle volume (ml) | 17.14 ± 1.79 | 16.28 ± 1.89 | 18.47 ± 2.11 | 18.62 ± 1.94 | 17.08 ± 1.83 | 16.16 ± 2.13 | 0.283 |
| Body mass index ($kg/m^2$) | 26.83 ± 1.82 | 26.25 ± 1.94 | 27.59 ± 1.88 | 28.16 ± 2.91 | 27.63 ± 2.68 | 25.46 ± 2.64 | 0.438 |

with normal morphology in medium- and higher-level treatment group were significantly higher than that in the medium- and higher-level placebo group respectively ($p < 0.05$). However, there were no significant changes at the first month and the second month of treatment ($p > 0.05$). It is worth to mention that there were no significant differences between lower-level treatment group and lower-level placebo group in terms of seminal parameters after at the third month of treatment ($p > 0.05$) (Table 2 and 3).

## 3.2 | Biochemical analysis

There were no significant differences in basal concentrations of FSH, inhibin B and T among all the groups ($p > 0.05$). At the third month of treatment, the circulating inhibin B in medium-level treatment group was significantly higher than that in medium-level placebo group ($p < 0.05$). However, no significant changes were observed at the first month and the second month of treatment ($p > 0.05$). It is worth noting that significant increases were observed in circulaing T in medium- and higher-level treatment groups in comparison with that in medium- and higher-level placebo groups respectively at the end of treatment ($p < 0.05$).

At the end of 3 months of treatment, there was a significant decrease in concentration of inhibin B; however, at the first month and second month of treatment, the concentration of inhibin B did not significantly decrease compared with that at baseline (Table 4).

**TABLE 2** The sperm count and total motile sperm number in each group

| Group | Baseline | First month of treatment | Second month of treatment | Third month of treatment |
|---|---|---|---|---|
| Sperm count (mil/ml) | | | | |
| Placebo group | | | | |
| Lower level | 11.82 ± 1.96 | 12.56 ± 1.87 | 12.85 ± 1.91 | 11.67 ± 1.89 |
| Medium level | 11.74 ± 1.83 | 12.51 ± 1.89 | 12.81 ± 1.94 | 11.48 ± 1.69 |
| Higher level | 12.23 ± 1.78 | 12.78 ± 1.67 | 11.88 ± 2.06 | 12.53 ± 2.13 |
| Treatment group | | | | |
| Lower level | 12.14 ± 1.97 | 11.63 ± 1.57 | 12.56 ± 2.13 | 13.84 ± 2.02 |
| Medium level | 11.57 ± 1.78 | 12.81 ± 1.94 | 13.23 ± 1.67 | 16.65 ± 2.21** |
| Higher level | 12.79 ± 1.86 | 12.21 ± 1.68 | 14.82 ± 1.98 | 18.63 ± 2.33† |
| Rate of forward motile spermatozoa (%) | | | | |
| Placebo group | | | | |
| Lower level | 25.38 ± 3.26 | 26.76 ± 3.04 | 24.87 ± 2.78 | 23.46 ± 3.26 |
| Medium level | 23.67 ± 2.65 | 24.49 ± 3.37 | 26.27 ± 2.95 | 23.87 ± 3.16 |
| Higher level | 26.49 ± 3.18 | 25.89 ± 3.85 | 23.38 ± 3.26 | 26.36 ± 3.14 |
| Treatment group | | | | |
| Lower level | 24.12 ± 2.69 | 27.12 ± 3.02 | 26.38 ± 3.32 | 26.38 ± 2.86 |
| Medium level | 25.94 ± 3.35 | 27.18 ± 3.53 | 28.12 ± 3.34 | 31.89 ± 3.68*,** |
| Higher level | 26.98 ± 3.26 | 25.89 ± 2.96 | 29.26 ± 3.45 | 34.12 ± 3.26***,† |
| Total motile sperm number (mil) | | | | |
| Placebo group | | | | |
| Lower level | 14.03 ± 1.76 | 15.67 ± 2.13 | 12.95 ± 2.52 | 14.96 ± 2.68 |
| Medium level | 12.95 ± 2.52 | 15.67 ± 2.13 | 14.18 ± 2.57 | 13.63 ± 2.21 |
| Higher level | 14.18 ± 2.57 | 12.57 ± 2.02 | 15.56 ± 1.39 | 13.87 ± 1.78 |
| Treatment group | | | | |
| Lower level | 13.26 ± 2.06 | 14.17 ± 2.51 | 12.19 ± 1.86 | 14.86 ± 2.38 |
| Medium level | 14.96 ± 2.19 | 16.31 ± 2.41 | 14.13 ± 2.15 | 19.89 ± 2.85*,** |
| Higher level | 12.24 ± 1.58 | 14.83 ± 2.25 | 16.17 ± 2.87 | 18.16 ± 2.52***,† |

*Indication of statistical significance ($p < 0.05$) when compare with that in medium-level placebo group after treatment assessed by analysis of variance. **Indication of statistical significance ($p < 0.05$) when compare with that in medium-level treatment group before treatment assessed by repeated-measures analysis of variance. ***Indication of statistical significance ($p < 0.05$) when compare with that in higher-level placebo group after treatment assessed by analysis of variance. †Indication of statistical significance ($p < 0.05$) when compare with that in higher-level treatment group before treatment assessed by repeated-measures analysis of variance.

**TABLE 3** The proportions of normally formed spermatozoa in each group

| Group | Baseline | First month of treatment | Second month of treatment | Third month of treatment |
|---|---|---|---|---|
| Proportions of normally formed spermatozoa (%) | | | | |
| Placebo group | | | | |
| Lower level | 7.03 ± 1.38 | 6.62 ± 1.06 | 7.19 ± 0.88 | 5.83 ± 0.91 |
| Medium level | 7.19 ± 0.98 | 5.93 ± 0.76 | 7.08 ± 1.37 | 6.81 ± 1.13 |
| Higher level | 6.62 ± 1.03 | 7.14 ± 1.16 | 5.87 ± 0.96 | 7.18 ± 1.06 |
| Treatment group | | | | |
| Lower level | 7.42 ± 1.32 | 6.17 ± 1.88 | 7.13 ± 1.28 | 6.43 ± 1.48 |
| Medium level | 6.81 ± 1.03 | 7.67 ± 1.65 | 7.34 ± 1.18 | 8.93 ± 1.86*,** |
| Higher level | 6.63 ± 0.89 | 7.33 ± 1.38 | 7.15 ± 1.23 | 9.43 ± 1.56***,† |

*Indication of statistical significance ($p < 0.05$) when compare with that in medium-level placebo group after treatment assessed by analysis of variance. **Indication of statistical significance ($p < 0.05$) when compare with that in medium-level treatment group before treatment assessed by repeated-measures analysis of variance. ***Indication of statistical significance ($p < 0.05$) when compare with that in higher-level placebo group after treatment assessed by analysis of variance. †Indication of statistical significance ($p < 0.05$) when compare with that in higher-level treatment group before treatment assessed by repeated-measures analysis of variance.

### 3.3 | Sperm DNA fragmentation

At the end of 3 months of treatment, there was a significant decrease in sperm DNA fragmentation index; however, at the first month and second month of treatment, the sperm DNA fragmentation index did not significantly decrease compared with that at baseline.

At baseline, there were nonsignificant differences in DNA fragmentation index among all the groups. At the end of treatment, the DNA fragmentation index in medium- and higher-level treatment groups were significantly lower than that in medium- and higher-level placebo group respectively ($p < 0.05$), whereas there were nonsignificant differences in the DNA fragmentation index between lower-level treatment group and lower-level placebo group ($p > 0.05$) (Table 5).

### 3.4 | Immunocytochemical staining

At the third month of treatment, spermatid concentration in medium- and higher-level treatment groups were significantly lower than that in medium- and higher-level placebo group respectively ($p < 0.05$), whereas there were nonsignificant differences in the DNA fragmentation index between lower-level treatment group and lower-level placebo group ($p > 0.05$) (Table 6).

### 3.5 | Spontaneous pregnancy rate

All the patients received a telephone call to identify clinical spontaneous pregnancy at day 90 after the end of treatment. The content of conversation included date of last normal menstrual period, serum hCG level and ultrasound confirmation of clinical pregnancy. Pregnancy testing was performed by the quantitative measurement of serum hCG level in the absence of menstruation. A clinical pregnancy was defined as the presence of a gestational sac and foetal heart rate motion on transvaginal ultrasound scanning. In this study, administration of combined hCG/hMG for 3 months could improve the rate of spontaneous pregnancy in medium- and higher-level group (32/112 and 3/16), in comparison with the rate of for placebo group (8/108 and 2/18) respectively ($p < 0.05$). Yet, there were nonsignificant differences in the rate of spontaneous pregnancy between lower-level treatment group and lower-level placebo group (7/32 and 6/30) ($p > 0.05$).

## 4 | DISCUSSION

Gonadotrophin administration in treating male suffering azoospermia or oligozoospermia with increased FSH level is generally regarded as useless (Lunenfeld et al., 1979). In the study of Knuth et al., 1987 thirty-nine severe oligospermia men have been recruited from a placebo-controlled, double-blinded trial, where sperm concentrations, total number of motile spermatozoa and the percentage of spermatozoa with normal morphology were similar in hCG/hMG-treated group and placebo-controlled group. However, Schill et al., 1982 demonstrated that combined hCH/hMG therapy in 48 subfertile male with idiopathic oligozoospermia was effective in a part of cases. Based on the above evidence, we designed this larger-sampled, randomised, placebo-controlled, double-blinded clinical study to evaluate the success of hCG/hMG therapy in male with idiopathic oligozoospermia in Chinese population and explore.

In this study, administration of hCG/hMG for 3 months could significantly improve the rate of sponaneous pregnancy in medium- and higher-level treatment groups (32/112 and 3/16), in comparison with the rate of placebo groups (8/108 and 2/18) respectively. However, there were nonsignificant differences in the rate of spontaneous pregnancy between lower-level treatment group and lower-level placebo group (7/32 and 6/30). Moreover, combined

**TABLE 4** Hormonal profiles before treatment, at the first month, the second month and the third month of treatment in each group

| Group | Baseline | First month of treatment | Second month of treatment | Third month of treatment |
|---|---|---|---|---|
| FSH (mIU/ml) | | | | |
| Placebo group | | | | |
| Lower level | 4.91 ± 0.54 | 4.58 ± 0.48 | 4.76 ± 0.53 | 4.43 ± 0.56 |
| Medium level | 4.82 ± 0.54 | 4.63 ± 0.51 | 4.23 ± 0.51 | 4.27 ± 0.50 |
| Higher level | 4.61 ± 0.54 | 4.98 ± 0.51 | 4.53 ± 0.51 | 4.83 ± 0.52 |
| Treatment group | | | | |
| Lower level | 5.36 ± 0.58 | 4.99 ± 0.61 | 5.29 ± 0.56 | 5.31 ± 0.58 |
| Medium level | 5.16 ± 0.58 | 5.33 ± 0.47 | 4.98 ± 0.59 | 5.25 ± 0.71 |
| Higher level | 4.86 ± 0.53 | 5.08 ± 0.62 | 4.85 ± 0.68 | 4.92 ± 0.54 |
| T (μg/L) | | | | |
| Placebo group | | | | |
| Lower level | 4.02 ± 0.45 | 4.09 ± 0.49 | 3.97 ± 0.32 | 4.17 ± 0.46 |
| Medium level | 4.14 ± 0.48 | 4.07 ± 0.42 | 4.18 ± 0.45 | 4.26 ± 0.53 |
| Higher level | 3.96 ± 0.43 | 3.89 ± 0.49 | 3.99 ± 0.42 | 4.04 ± 0.49 |
| Treatment group | | | | |
| Lower level | 3.92 ± 0.42 | 4.13 ± 0.57 | 3.94 ± 0.51 | 3.98 ± 0.49 |
| Medium level | 4.09 ± 0.54 | 4.16 ± 0.52 | 4.29 ± 0.49 | 5.37 ± 0.58*,** |
| Higher level | 4.07 ± 0.66 | 4.15 ± 0.54 | 4.31 ± 0.62 | 5.49 ± 0.59***,† |
| Inhibin B (pg/ml) | | | | |
| Placebo group | | | | |
| Lower level | 76.18 ± 9.23 | 78.84 ± 10.28 | 69.78 ± 8.42 | 74.83 ± 11.47 |
| Medium level | 148.63 ± 17.14 | 142.84 ± 16.28 | 156.13 ± 14.47 | 150.78 ± 18.42 |
| Higher level | 326.45 ± 34.69 | 323.13 ± 24.47 | 331.84 ± 46.28 | 318.76 ± 38.64 |
| Treatment group | | | | |
| Lower level | 78.81 ± 11.76 | 79.13 ± 12.38 | 86.82 ± 14.91 | 83.81 ± 12.76 |
| Medium level | 151.62 ± 16.26 | 158.32 ± 19.41 | 163.27 ± 18.42 | 188.74 ± 22.41*,** |
| Higher level | 328.16 ± 46.58 | 324.78 ± 38.42 | 335.78 ± 18.42 | 318.26 ± 31.89 |

*Indication of statistical significance ($p < 0.05$) when compare with that in medium-level placebo group after treatment assessed by analysis of variance. **Indication of statistical significance ($p < 0.05$) when compare with that in medium-level treatment group before treatment assessed by repeated-measures analysis of variance. ***Indication of statistical significance ($p < 0.05$) when compare with that in higher-level placebo group after treatment assessed by analysis of variance. †Indication of statistical significance ($p < 0.05$) when compare with that in higher-level treatment group before treatment assessed by repeated-measures analysis of variance.

hCG/hMG therapy for 3 months resulted in statistically significant improvements in such semen parameters as sperm concentration, rate of forward motile spermatozoa, total motile sperm count and the percentage of spermatozoa with normal morphology in medium- and higher-level treatment group. Spermatogenesis is categorised into three phases: mitosis (spermatogonia), meiosis (spermatocytes) and spermiogenesis (the morphological transformation of spermatids) (McLachlan et al., 2002). These phases involve the coordinated proliferation, differentiation and survival of progressively maturing germ cells which are regulated by LH and FSH (Farmakiotis et al., 2007; You et al., 2017). The improvement in testicular function observed in this study could be due to the potential beneficial effect of hCG/hMG therapy on Leydig cells and Sertoli cells.

The results of this study demonstrated that at the first month and second month of treatment, the sperm concentration, rate of forward motile spermatozoa, total number of motile spermatozoa and the percentage of spermatozoa with normal morphology did not significantly increase compared with that at baseline. For human, the entire process of spermatogenesis is estimated as taking approximately 3 months (You et al., 2017). In this study, 3 months of treatment of combined hCG/hMG has a beneficial effect on infertile male with idiopathic oligozoospermia in terms of semen parameters, while the treatment for a period of <3 months is not effective; therefore, the result indicates that the duration of the treatment is at least 3 months.

Circulating inhibin B level correlates directly with spermatogenesis and reflect testicular sperm production (Hamdi, Almont, Galinier, Mieusset, & Thonneau, 2017). Inhibin B secretion has been regarded as a reliable index of Sertoli cell function (de Kretser, Loveland, Meinhardt, Simorangkir, & Wreford, 1998). This randomised, blinded

**TABLE 5** Sperm DNA fragmentation before treatment, at the first month, the second month and the third month of treatment in each group

| Group | Baseline | First month of treatment | Second month of treatment | Third month of treatment |
|---|---|---|---|---|
| DNA fragmentation (%) | | | | |
| Placebo group | | | | |
| Lower level | 18.68 ± 2.09 | 17.98 ± 2.45 | 18.13 ± 1.91 | 19.12 ± 2.16 |
| Medium level | 18.36 ± 1.98 | 18.61 ± 2.06 | 17.96 ± 1.91 | 18.91 ± 2.42 |
| Higher level | 18.12 ± 2.21 | 17.47 ± 2.12 | 17.99 ± 1.34 | 18.06 ± 2.31 |
| Treatment group | | | | |
| Lower level | 19.86 ± 2.48 | 17.99 ± 1.91 | 18.29 ± 2.16 | 19.12 ± 2.54 |
| Medium level | 18.13 ± 1.94 | 16.33 ± 2.14 | 16.98 ± 2.29 | 13.26 ± 1.68*,** |
| Higher level | 17.68 ± 2.13 | 15.98 ± 1.62 | 16.15 ± 1.86 | 14.92 ± 1.52***,† |

*Indication of statistical significance ($p < 0.05$) when compare with that in medium-level placebo group after treatment assessed by analysis of variance. **Indication of statistical significance ($p < 0.05$) when compare with that in medium-level treatment group before treatment assessed by repeated-measures analysis of variance. ***Indication of statistical significance ($p < 0.05$) when compare with that in higher-level placebo group after treatment assessed by analysis of variance. †Indication of statistical significance ($p < 0.05$) when compare with that in higher-level treatment group before treatment assessed by repeated-measures analysis of variance.

**TABLE 6** Spermatid and leucocyte concentration before treatment, at the first month, the second month and the third month of treatment in each group

| Group | Baseline | First month of treatment | Second month of treatment | Third month of treatment |
|---|---|---|---|---|
| Spermatid concentration (mil/ml) | | | | |
| Placebo group | | | | |
| Lower level | 1.28 ± 0.17 | 1.68 ± 0.26 | 1.26 ± 0.24 | 0.93 ± 0.21 |
| Medium level | 1.38 ± 0.26 | 1.18 ± 0.15 | 1.04 ± 0.16 | 1.26 ± 0.18 |
| Higher level | 1.24 ± 0.19 | 0.97 ± 0.23 | 1.28 ± 0.26 | 1.08 ± 0.21 |
| Treatment group | | | | |
| Lower level | 1.28 ± 0.19 | 1.06 ± 0.15 | 0.97 ± 0.23 | 1.08 ± 0.17 |
| Medium level | 1.36 ± 0.21 | 0.98 ± 0.26 | 1.12 ± 0.20 | 0.68 ± 0.16*,** |
| Higher level | 1.16 ± 0.17 | 0.96 ± 0.31 | 1.06 ± 0.19 | 0.62 ± 0.12***,† |
| Leucocyte concentration (mil/ml) | | | | |
| Placebo group | | | | |
| Lower level | 0.48 ± 0.16 | 0.53 ± 0.18 | 0.61 ± 0.21 | 0.46 ± 0.17 |
| Medium level | 0.62 ± 0.18 | 0.51 ± 0.14 | 0.58 ± 0.16 | 0.68 ± 0.21 |
| Higher level | 0.57 ± 0.12 | 0.60 ± 0.13 | 0.49 ± 0.11 | 0.53 ± 0.09 |
| Treatment group | | | | |
| Lower level | 0.60 ± 0.16 | 0.58 ± 0.15 | 0.68 ± 0.09 | 0.47 ± 0.12 |
| Medium level | 0.53 ± 0.08 | 0.57 ± 0.13 | 0.48 ± 0.16 | 0.61 ± 0.11 |
| Higher level | 0.48 ± 0.09 | 0.65 ± 0.16 | 0.62 ± 0.14 | 0.48 ± 0.11 |

*Indication of statistical significance ($p < 0.05$) when compare with that in medium-level placebo group after treatment assessed by analysis of variance. **Indication of statistical significance ($p < 0.05$) when compare with that in medium-level treatment group before treatment assessed by repeated-measures analysis of variance. ***Indication of statistical significance ($p < 0.05$) when compare with that in higher-level placebo group after treatment assessed by analysis of variance. †Indication of statistical significance ($p < 0.05$) when compare with that in higher-level treatment group before treatment assessed by repeated-measures analysis of variance.

study demonstrates that combined hCG/hMG therapy stimulates spermatogenesis and increases sperm concentration only when circulating inhibin B level is in the medium- and higher-level. It implies that combined hCG/hMG treatment is not effective in unselected oligozoospermic men, underlying the importance of criteria for the selection of patients to be treated with hCG/hMG. The significant increase in inhibin B concentration in part of the patients (medium-level of inhibin B) is attributed to the potential beneficial effect of

combined hCG/hMG therapy on the spermatogenesis function. There were no significant differences in inhibin B in lower- and higher-level group before and after treatment. Furthermore, a significant increase was observed in circulating T level in medium- and higher-level treatment group in comparison with that in placebo group. Elevated circulating T level may be due to the stimulation of hCG/hMG on Leydig cells.

The DNA fragmentation index is recognised as a marker for sperm quality and a diagnostic tool in fertile men, considering that high sperm DNA fragmentation index is associated with poor ART outcomes (Gandini et al., 2000). There are several intrinsic and extrinsic causes of sperm DNA damage. Endogenous causes include protamine deficiency, ageing and oxidative stress. Known exogenous causes of DNA damage include heat, inflammation of the genital tract and hormonal imbalance (Simon, Castillo, Oliva, & Lewis, 2011). Sperm DNA fragmentation originates mostly in the testis resulting from an apoptotic mechanism or from oxidative stress during transit in the male genital tract (Simon et al., 2011). We hypothesises that combined hCG/hMG administration decreases sperm fragmentation index by reducing apoptosis or/and oxidative stress (Muratori & Baldi, 2018; Surico et al., 2015).

Normal human semen may contain, in addition to spermatozoa, round nucleated germ cells, leucocytes and other cells (e.g., macrophages and epithelial cells). The presence of spermatogenic cells in semen samples suggests a testicular malfunction where the impaired spermatogenesis could be of endocrinological origin (Johanisson, Campana, Luthi, & Agostini, 2000). Increased number of immature germ cells might play a pivotal role in the pathogenesis of abnormal spermatozoa. In this study, we used immunocytochemical staining with monoclonal antibodies to the human intra-acrosomal SP-10 antigen and the common CD-45 human leucocyte antigen to assist in cellular identification in human semen (Foster & Herr, 1992). In the present experiment, combined hCG/hMG treatment significantly decreased spermatid concentration. It supports the speculation that hCG/hMG therapy improves spermatogenesis.

A body of evidence has proved that administration of FSH exerts beneficial effects on idiopathic oligozoospermia (Valenti et al., 2013); however, FSH was administrated by abdominal wall injection daily and highly purified FSH/recombinant hFSH was relatively expensive; hCG/hMG therapy is therefore suggested as an alternative to therapy with FSH.

In summary, the present experiment shows that combined hCG/hMG therapy for 3 months could result in statistically significant improvements in a part of males with idiopathic oligozoospermia in terms of seminal parameters, DNA fragmentation and spontaneous pregnancy rate, and which may mediated by increased inhibin B secretion and possibly a beneficial effect of on the testes. The efficacy of hCG/hMG therapy is associated with the inhibin B level.

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## ORCID

*Jian-Min Zhang* https://orcid.org/0000-0003-4054-665X

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