

## Acupuncture Research

# Effect of Electroacupuncture on Spermatogenesis in Rats with Oligozoospermia of Insufficiency of Shen (Kidney) Essence Syndrome\*

CUI Tian-wei<sup>1</sup>, QIN Mao<sup>2</sup>, LIU Bao-xing<sup>1</sup>, GAO Yun-xiao<sup>1</sup>, MA Wen-jing<sup>1</sup>, and ZHANG Xiu-ping<sup>1</sup>

**ABSTRACT** **Objective:** To assess the effect of electroacupuncture (EA) on expression of cytoskeletal proteins from Sertoli cells (SCs) and spermatogenesis in rats with oligozoospermia of insufficiency of Shen (Kidney) essence syndrome (OIKES). **Methods:** Twenty healthy male Sprague-Dawley rats were randomly assigned to four groups using a random number table: control, tripterygium glycosides (TG) treatment, sham and EA groups ( $n=5$  in each group). A rat model of OIKES was established by oral gavage with TG. The EA group was treated with TG and received EA at Shenshu (BL 23) and Zusanli (ST 36) acupoints for 20 min, once daily for 30 days, while the sham group received EA at identical acupoints with skin penetration without stimulation. After 30 days, the final body weight and coefficients for the testis and epididymis were calculated and sperm parameters were measured. Immunohistochemical analyses were performed to detect expression of vimentin and  $\alpha$ -tubulin in SCs and proliferating cell nuclear antigen (PCNA) immunoreactivity in germ cells. Apoptosis in germ cells was quantified by the transferase biotin-dUTP nick end labeling assay. **Results:** Compared with the control group, the final body weight and testis/epididymis coefficients of rats in the TG-treated group were not significantly different, but the sperm count and motility were lower ( $P<0.05$ ). Expressions of vimentin and  $\alpha$ -tubulin were also significantly weaker ( $P<0.01$ ). The PCNA immunoreactivity of germ cells was decreased ( $P=0.059$ ), whereas the apoptotic index of germ cells was increased significantly ( $P<0.01$ ). In contrast, EA at BL 23 and ST 36 acupoints significantly improved the final body weight as well as the sperm count, concentration and motility ( $P<0.01$  or  $P<0.05$ ). EA increased expression of vimentin and  $\alpha$ -tubulin in SCs markedly, and significantly enhanced PCNA immunoreactivity with decreased apoptosis in germ cells ( $P<0.01$  or  $P<0.05$ ). **Conclusions:** EA at BL 23 and ST 36 acupoints has protective effects on spermatogenesis in rats with OIKES. This effect seems to be achieved by attenuating TG-induced disruption of cytoskeletal protein in SCs.

**KEYWORDS** electroacupuncture, Sertoli cells, cytoskeletal proteins, oligozoospermia, insufficiency of Shen (Kidney) essence syndrome

Sertoli cells (SCs) are in close contact with germ cells in the seminiferous epithelium and are called the "nurse cells" of spermatogenic cells. They undertake essential endocrine and paracrine duties, and provide nutritional and physical support to germ cells during spermatogenesis.<sup>(1-3)</sup> The cytoskeleton of healthy SCs plays a key role in the maintenance of normal spermatogenesis, including ensuring the structural integrity of seminiferous epithelium, moving elongating spermatids during the seminiferous epithelial cycle, as well as the adhesion and release of elongated spermatids. Physical or chemical alterations in the cytoskeleton of SCs hamper spermatogenesis severely.<sup>(4,5)</sup>

Administration of tripterygium glycosides (TG) is a commonly used treatment for rheumatoid

arthritis. The TG-treated rat model has been used to study spermatogenesis successfully, and is an established model for insufficiency of Kidney (Shen) essence syndrome (OIKES) in Chinese medicine.<sup>(6)</sup> Moreover, SCs are the targets of TG. Previously, We demonstrated that TG has detrimental effects on the

© The Chinese Journal of Integrated Traditional and Western Medicine Press and Springer-Verlag GmbH Germany, part of Springer Nature 2018

\*Supported by the National Natural Science Foundation of China (No. 81273767 and 81473690)

1. Department of Andrology, China-Japan Friendship Hospital, Beijing (100029), China; 2. Department of Andrology, Chongqing Health Center for Women and Children, Chongqing (400013), China

Correspondence to: Dr. LIU Bao-xing, Tel: 86-10-84205109, E-mail: [liubx66@163.com](mailto:liubx66@163.com)

DOI: <https://doi.org/10.1007/s11655-018-2915-9>

secretory function of SCS.<sup>(7)</sup>

Acupuncture is one of several methods of Chinese medicine (CM) used to treat male infertility.<sup>(8)</sup> Increasing evidence suggests that electroacupuncture (EA) can improve the sperm count, motility, and morphology in subfertile men (at least in part) by modulation of SCS function.<sup>(9,10)</sup> However, the mechanism underlying these effects is understood incompletely. The purpose of the present study is to investigate the effects of EA on the protein expression of SCS cytoskeleton and spermatogenesis in TG-treated rats.

## METHODS

### Animals and Experimental Design

The Ethics Committee of China-Japan Friendship Hospital (Beijing, China) approved the study protocol. Twenty healthy male and specific-pathogen free (SPF) grade Sprague-Dawley rats (8 weeks, 250–300 g) were obtained from Beijing Vital River Experimental Animal Center [Beijing, China, experiment animal certificate No. SCXK (JING) 2012-0001]. All rats were housed in a SPF laboratory at  $23 \pm 1$  °C, humidity 55%–60%, 12 h light/dark cycle in the Experimental Animal Center of China-Japan Friendship Hospital [certification No. SYCK (Jing) 2015-0017]. Before experimentation, rats were fed adaptively for 7 days with a standard diet and free access to water.

Rats were randomly assigned to four groups using a random number table ( $n=5$  each), the body weight had no statistically significant difference among 4 groups. In addition to normal feeding, the control group underwent daily gavage with 50 mg/(kg·d) carboxymethyl cellulose and the TG-treated group underwent gavage with 20 mg/(kg·d) TG, for 30 days.<sup>(11)</sup> The EA group received stimulation to bilateral Shenshu (BL 23) and Zusanli (ST 36) acupoints (selected on the basis of Experimental Acupuncture Science<sup>(12)</sup>). Rats were fixed in a customized cage, and acupuncture needles inserted at depths of approximately 6 mm at two acupoints, BL 23 (located on the sacrum, medial and inferior to the posterosuperior iliac spine, just at the second posterior sacral foramen) and ST 36 (located near the knee joint, 5 mm lateral to the anterior tubercle of the tibia). EA stimulation was at 1 mA intensity and 10 Hz frequency using a Huatuo EA instrument (100 A; Suzhou Medical Devices, Suzhou, China). EA treatment was applied for 20 min, once daily, and continuously for 30 days. Acupuncture needles were inserted superficially (about 1 mm) at identical locations,

but without stimulation or needle retention in the sham group. The frequency of treatment was the same as that in the EA group.

### Measurement of Final Body Weight, Testis/Epididymis Coefficients, and Sperm Parameters in Rats

Rats were sacrificed with the method of cervical dislocation after weighing, then testis and epididymis tissues were harvested for analysis. The cauda epididymis of the rat testis was minced and suspended in M199 medium at 37 °C for 10 min. Next, the sperm suspensions were placed on a marker chamber. The sperm count, concentration and motility were measured by a computer-aided sperm analysis system (Hamilton-Thorne Research, Beverly, MA, USA).

### Immunohistochemical Analyses

#### Vimentin and $\alpha$ -Tubulin

Specimens of testis tissue were embedded in paraffin and sectioned (thickness, 4  $\mu$ m). After sections had been deparaffinized in water, they were placed in sodium citrate solution in a microwave oven at moderate heat for 10 min for antigen retrieval. After cooling naturally, the sections were incubated in 3% H<sub>2</sub>O<sub>2</sub> for 10 min and then blocked with bovine serum albumin (Solarbio, Beijing, China) for 30 min at room temperature. Tissue sections were then incubated with antibodies to vimentin (1:100 dilution, rabbit, Abcam, Cambridge, UK) and  $\alpha$ -tubulin (1:200 dilution, rabbit, Abcam, Cambridge, UK) overnight at 4 °C. Positive cells were detected using peroxidase-conjugated streptavidin followed by 3'3-diaminobenzidine tetrahydrochloride (DAB; Beijing Zhongke Wanbang Biological Technology, Beijing, China) staining. The slides were counterstained with hematoxylin. Finally, the sections were dehydrated in a graded series of ethanol solutions, cleared in xylene and cover-slipped and mounted with neutral resin. Slides were observed under a light microscope (BX51; Olympus, Tokyo, Japan) and the mean densities of vimentin and  $\alpha$ -tubulin in 100 microscopic fields calculated with image-processing software (Image-Pro Plus 6.0; Media Cybernetics, Rockville, MD, USA).

#### Proliferating Cell Nuclear Antigen

To evaluate proliferating cells, tissue blocks were cut into 4- $\mu$ m sections. Briefly, sections were dewaxed in xylene followed by rehydration in decreasing grades of ethanol. Sections were treated with 0.3% H<sub>2</sub>O<sub>2</sub> in methanol to block endogenous peroxidase activity.

Non-specific binding sites were blocked using 1% normal goat serum for 30 min. Primary antibody against proliferating cell nuclear antigen (PCNA, Histostain® -Plus Mouse Primary; Invitrogen, Carlsbad, CA, USA) was added to the sections, followed by overnight incubation at 4 °C. To probe primary antibody binding sites, biotinylated universal secondary antibody (Histostain-Plus Rabbit Primary, Invitrogen Carlsbad, CA, USA) was added for 30 min. Positive cells were revealed with DAB chromogen (DAB kit, Invitrogen, Carlsbad, CA, USA). Counterstaining was carried out with hematoxylin, after which cells were mounted with Entellan® (Millipore, Billerica, MA, USA). Slides were observed under a light microscope (BX41, Olympus) at 200 × magnification. PCNA as a marker of cell proliferation was calculated as the mean density of PCNA-positive cells per tubule; 100 tubular cross-sections chosen randomly were evaluated for each slide using image processing software (Image-Pro Plus).

**Detection of Apoptosis**

The terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay was carried out using an *in situ* cell death detection kit (Roche, Basel, Switzerland) to detect apoptosis. According to manufacturer instructions, paraffin-embedded tissue sections were dewaxed in xylene, rehydrated through a graded series of ethanol solutions, and pretreated with proteinase-K. Endogenous peroxidase activity was blocked by immersion in 3% H<sub>2</sub>O<sub>2</sub> in methanol for 10 min. Each slice was stained with DAB, and apoptosis of germ cells was observed under light microscopy. The number of TUNEL-positive cells per tubule was counted in 100 random tubular cross-sections under 200 × magnification for each slide, and percentages of positive cells were averaged with image processing software (Image-Pro Plus).

**Statistical Analyses**

Results are presented as mean ± standard deviation ( $\bar{x} \pm s$ ) for normally distributed data and

median (upper quartile–lower quartile) for non-normally distributed data. Statistical analyses were carried out by one-way analysis of variance (ANOVA) for normally distributed data and Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison post hoc tests on ranks for skewed data with SPSS software version 20.0 (IBM, Armonk, NY, USA). *P*<0.05 was considered significant.

**RESULTS**

**Effect of EA on Final Body Weight, Testis/Epididymis Coefficients, and Sperm Parameters**

Compared with the control group, the final body weight slightly decreased and the testis/epididymis coefficients slightly increased in the TG-treated group, respectively (*P*>0.05), meanwhile the sperm count and motility significantly declined (*P*<0.05). Compared with the TG-treated group, the final body weight slightly increased in the EA group (*P*>0.05), meanwhile the sperm motility slightly increased (*P*=0.058) and the sperm count and concentration were significantly improved (*P*<0.05). The sham group showed no-significant variation compared with the TG-treated group in terms of final body weight, testis/epididymis coefficients, and sperm parameters (Table 1).

**Expression of Vimentin and α-Tubulin in SCs**

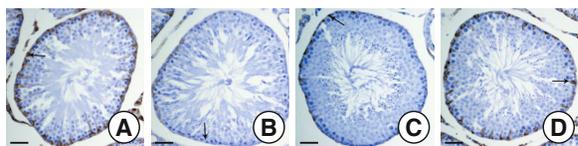
Vimentin expression level was significantly decreased in the TG-treated group [3.07 (1.60, 5.34)] compared with the control group [31.69 (19.47, 44.68, *P*<0.01)]. Compared with the TG-treated group, vimentin expression level was significantly increased in the EA group [18.91 (10.19, 28.07), *P*<0.01] and the sham group [6.76 (2.95, 22.12), *P*<0.01, Figure 1], but the level of EA group was significantly higher than that of the sham group (*P*<0.01).

The TG-treated group showed a significant reduction in α-tubulin expression level compared with the control group (*P*<0.01). Compared with the TG-treated group, α-tubulin expression level was not obviously different in the sham group, but the level of α-tubulin expression was significantly enhanced in

**Table 1. Effect of EA on Final Body Weight, Testis/Epididymis Coefficients, and Sperm Parameters ( $\bar{x} \pm s$ )**

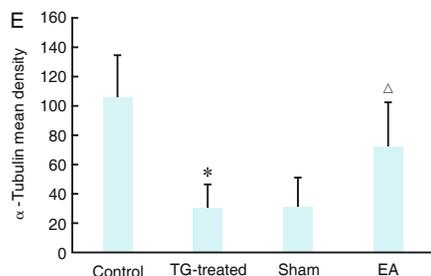
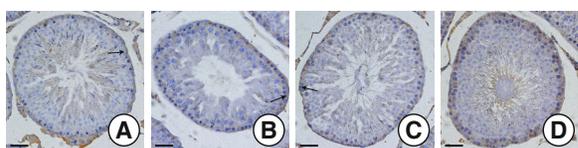
Group	n	Final body weight (g)	Testis coefficient (mg/g)	Epididymis coefficient (mg/g)	Sperm count (× 10 <sup>6</sup> )	Sperm concentration (× 10 <sup>6</sup> /mL)	Sperm motility (%)
Control	5	455.20 ± 18.21	8.07 ± 0.81	2.88 ± 0.22	58.14 ± 21.39	19.20 ± 8.99	59.60 ± 10.66
TG-treated	5	428.80 ± 10.20	8.69 ± 0.44	2.96 ± 0.24	50.86 ± 19.32*	18.38 ± 7.75	53.60 ± 22.54*
Sham	5	439.40 ± 24.57	7.96 ± 0.61	2.82 ± 0.17	65.88 ± 13.37	21.78 ± 3.88	62.20 ± 6.90
EA	5	457.40 ± 35.26	8.18 ± 1.13	2.95 ± 0.37	128.36 ± 69.73 <sup>Δ</sup>	44.80 ± 26.03 <sup>Δ</sup>	71.40 ± 9.65

Notes: \**P*<0.05, compared with the control group; <sup>Δ</sup>*P*<0.05, compared with the TG-treated group



**Figure 1. Effect of EA on Vimentin Expression in SCs by Immunohistochemistry (DAB staining  $\times 200$ )**

Notes: Vimentin expression was visualized by immunohistochemistry. Arrows indicate positive staining. In the control group, vimentin filaments surrounded the nucleus and extended from the basal region towards the lumen (A). A dramatic loss of vimentin expression was detected after TG treatment, whereas spermatogenic cells became detached from SCs and sloughed into the lumen (B). Sham group had a non-significant impact on vimentin expression (C), whereas EA treatment increased vimentin expression (D). Scale bar=20.



**Figure 2. Effect of EA on  $\alpha$ -Tubulin Expression in SCs by Immunohistochemistry ( $\times 200$ )**

Notes:  $\alpha$ -Tubulin expression was visualized by immunohistochemistry. Arrows indicate positive staining of  $\alpha$ -tubulin. In the control group, the  $\alpha$ -tubulin staining pattern was characterized by tracts extending along axes of the SCs (A). Staining was weak in the TG-treated group, indicating that TG exposure disrupted  $\alpha$ -tubulin expression in SCs (B). Differences in  $\alpha$ -tubulin expression in the sham group were not obvious (C), but  $\alpha$ -tubulin expression was enhanced near the basal region following EA treatment (D). Quantity analysis of  $\alpha$ -tubulin mean density ( $\bar{x} \pm s$ ,  $n=5$  in each group (E)). Scale bar=20  $\mu$  m. \* $P<0.05$ , compared with the control group;  $\triangle P<0.01$ , compared with the TG-treated group

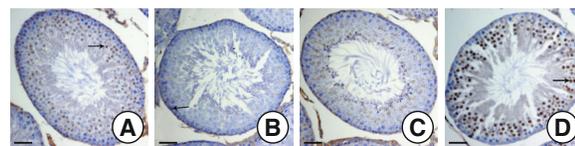
the EA group ( $P<0.01$ , Figure 2).

### Immunoreactivity of PCNA in Germ Cells

The weaker PCNA immunoreactivity was observed in the TG-treated group [9751.81 (5638.12, 16977.02)] compared with the control group [12092.35 (8081.04, 23071.47,  $P=0.059$ )]. Compared with the TG-treated group, a significant difference was observed in the sham group [12.48.85 (9148.33, 16384.80),  $P<0.05$ ], but stronger PCNA immunoreactivity was found in the EA group [13750.55 (8840.05, 19048.75),  $P<0.01$ , Figure 3].

### TUNEL Assay

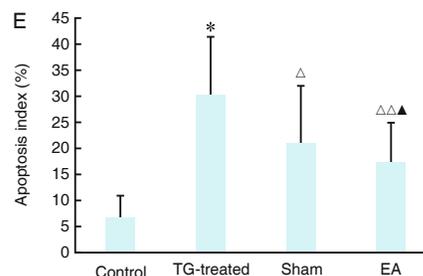
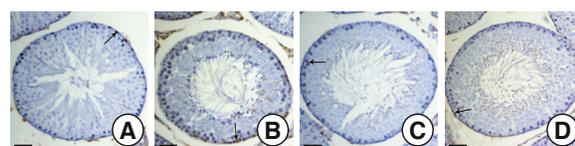
The effect of EA treatment on the apoptosis



**Figure 3. Effect of EA on PCNA Immunoreactivity in Germ Cells ( $\times 200$ )**

Notes: Arrows indicate PCNA-positive germ cells. The germ cells were typically present in the seminiferous tubules in the control group (A), whereas the TG-treated group showed weaker PCNA immunoreactivity and fewer spermatogenic cells in the seminiferous tubules (B). The intensity of PCNA immunoreactivity and the number of spermatogenic cells were higher in both sham and EA groups. Compared with the sham group, EA treatment showed stronger PCNA immunoreactivity with an increased number of germ cells in the seminiferous tubules (C–D). Scale bar=20  $\mu$  m.

of germ cells was assessed by TUNEL staining. Compared with the control group, a significantly higher apoptosis index (AI) was observed in the TG-treated group ( $P<0.05$ ). The sham group and EA group had a lower AI ( $P<0.01$  and  $P<0.05$ , respectively), but the EA group appeared much lower AI compared with the sham group ( $P<0.05$ , Figure 4).



**Figure 4. Effect of EA on AI in Germ Cells by TUNEL Assay ( $\times 200$ )**

Notes: TUNEL analyses for apoptotic spermatogenic cells in the control (A), TG-treated (B), sham (C), and EA (D) groups. Arrows indicate apoptotic cells. Qualitatively, TUNEL staining was more intense in the cell nuclei of the TG-treated group than in the control group (A–B). Less apoptotic cells were noted in the sham group (C). Germ cells in the EA group showed limited apoptosis and relatively intact nuclear membranes (D). Quantity analysis of AI ( $\bar{x} \pm s$ ,  $n=5$  in each group (E)). \* $P<0.05$ , compared with the control group;  $\triangle P<0.01$ ,  $\triangle\triangle P<0.01$ , compared with the TG-treated group;  $\blacktriangle P<0.05$ , compared the sham group. AI values represent the number of apoptotic cells/total germ cells in the seminiferous tubules. Scale bar= 20  $\mu$  m.

## DISCUSSION

According to basic CM theory, the Shen dominates reproduction in the human body. Shen essence deficiency syndrome is manifested mainly as reduced libido, soreness and weakness of the waist and knees, dizziness, tinnitus and "spiritual burnout".<sup>(13)</sup> The

coefficients of testis/epididymis are important indicators to evaluate the function of spermatogenesis. However, due to changes in final body weight of rats after intervention, differences of testis/epididymis coefficients were observed insignificant in the four groups. The behavior of TG-treated rats indicated that we had successfully established a rat model of insufficiency of Shen essence syndrome.

Recent work has suggested that TG can inhibit the function and apoptosis of germ cells by inducing the formation of reactive oxygen species and inhibiting the antioxidant defense systems.<sup>(14)</sup> Impaired spermatogenesis might be caused by abnormal metabolism of lipids and energy in the testis via TG-mediated down-regulation of peroxisome proliferator-activated receptors.<sup>(15)</sup> Previously, we demonstrated that TG can suppress spermatogenesis by inhibiting the expression of secretory proteins from SCs.<sup>(7)</sup> Here, we showed that TG could suppress spermatogenesis and decreased sperm production in rats significantly. Furthermore, TG treatment disrupted the expression of cytoskeletal proteins. These effects were accompanied by the induction of apoptosis and reduced PCNA immunoreactivity in germ cells.

Acupuncture is an effective therapy for improving sperm parameters.<sup>(16)</sup> The acupoints we selected are used commonly in research and clinical practice related to the reproductive system. CM theory states that Shen is the root of the congenital constitution and Pi (Spleen) is the root of the acquired constitution. BL 23 denotes the shen Back-Shu points that represent the Shen vital essence infusion to the body back surface of the body, ST 36 is the key point for regulation of the Pi and Wei (Stomach). Therefore, BL 23 and ST 36 are the most commonly used acupoints to treat infertility because they tonify Kidney and strengthen essence. Studies have shown that acupuncture at BL 23 and ST 36 acupoints is associated with a higher prevalence of pregnancy in patients who undergo *in vitro* fertilization embryo transfer or those with infertility due to ovulatory disturbances.<sup>(17,18)</sup> Acupuncture also has clear therapeutic effects in patients with low sperm count due to inflammation of the genital tract caused by scrotal hyperthermia, as well as male immune infertility.<sup>(9,19)</sup>

The mechanism of acupuncture on improving infertility is still unclear.<sup>(20)</sup> Accumulating evidence suggests that acupuncture might improve the sperm

count,<sup>(9)</sup> motility<sup>(10)</sup> and the prevalence of fertilization after intracytoplasmic sperm injection in subfertile men,<sup>(21)</sup> at least in part, by modulating levels of follicle-stimulating hormone, luteinizing hormone, and testosterone.<sup>(22)</sup> It is thought that acupuncture enhances germ cell proliferation by improving SC function, thereby facilitating the recovery of spermatogenesis.<sup>(16)</sup> EA enhances germ cell proliferation and restores normal levels of inhibin B presumably by improving the functions of SCs and Leydig cells.<sup>(1)</sup> However, little is known about how acupuncture enhances spermatogenesis from the perspective of the SC cytoskeleton.

Vimentin has been reported to have significant roles in the integrity of cells and tissue.<sup>(23)</sup> Damage to the SC cytoskeleton leads to germ cell apoptosis, sperm cell deformation, and migration abnormalities.<sup>(24)</sup>  $\alpha$ -Tubulin is another cytoskeletal protein that is a major component of microtubules; it regulates intracellular transport as well as the shape, motility, migration, and division of cells.<sup>(25,26)</sup> In our study, TG induced the collapse of vimentin filaments in SCs, which may have disrupted physical interactions between SCs and spermatogenic cell, and induced apoptosis of the latter.

Acupuncture has been shown to affect cytoskeletal remodeling in subcutaneous connective tissue.<sup>(27)</sup> In addition, EA on acupoints of the Wei Meridian could promote repair of the injured gastric mucosa, which might be related to its effects in regulating the levels of cytoskeletal proteins.<sup>(28)</sup> Our results showed a gradual collapse of vimentin filaments and decreased intensity of  $\alpha$ -tubulin staining in the SCs of TG-treated rats, whereas spermatogenic cells became separated from basement membranes and sloughed into the lumen compared with controls. We speculate that TG-induced disruption of vimentin filaments in SCs may lead to premature SC release of spermatogenic cells, which might undergo the apoptosis following loss of SC support. We examined the effects of acupuncture on spermatogenesis and sperm parameters using rats with TG-treated testes. EA exhibited a protective effect on spermatogenesis as shown by higher final body weight, sperm count, sperm concentration and sperm motility. These improvements after EA were associated with disrupted expression of cytoskeletal proteins of SCs and enhanced proliferation of germ cells (PCNA staining) with reduced apoptosis of germ cells.

In conclusion, EA at BL 23 and ST 36 acupoints

protected the germ cells of rats with OIKES. EA facilitated cytoskeletal remodeling by up-regulating expression of vimentin and  $\alpha$ -tubulin in SCs. However, the specifically mechanism of EA on improving spermatogenesis requires further study.

### Conflict of Interest

The authors declare that they have no conflict of interest.

### Author Contribution

Liu BX, Cui TW, and Qin M conceived and designed the experiments. Cui TW, Qin M, Gao YX, Ma WJ, and Zhang XP performed the experiments. Cui TW analyzed the data and wrote the manuscript.

## REFERENCES

- Dimitriadis F, Tsiampali C, Chaliasos N, Tsounapi P, Takenaka A, Sofikitis N. The Sertoli cell as the orchestra conductor of spermatogenesis: spermatogenic cells dance to the tune of testosterone. *Hormones (Athens)* 2015;14:479-503.
- Svingen T, Koopman P. Building the mammalian testis: origins, differentiation, and assembly of the component cell populations. *Genes Dev* 2013;27:2409-2426.
- Hermo L, Pelletier RM, Cyr DG, Smith CE. Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 5: intercellular junctions and contacts between germ cells and Sertoli cells and their regulatory interactions, testicular cholesterol, and genes/proteins associated with more than one germ cell generation. *Microsc Res Tech* 2010;73:409-494.
- Vogl AW, Vaid KS, Guttman JA. The Sertoli cell cytoskeleton. *Adv Exp Med Biol* 2008;636:186-211.
- Johnson KJ. Testicular histopathology associated with disruption of the Sertoli cell cytoskeleton. *Spermatogenesis* 2014;4:e979106.
- Mao PM, Li HS, Wang B, Mo XW. Progress in research on preparation of animal model of tripterygium glycoside induced infertility. *Chin J Integr Tradit Chin Med (Chin)* 2015;35:254-256.
- Xu YP, Liu BX, Zhang XP, Yang CW, Wang CH. A Chinese herbal formula, Wuzi Yanzong Pill, improves spermatogenesis by modulating the secretory function of Sertoli cells. *Chin J Integr Med* 2014;20:194-199.
- Qu F, Li R, Sun W, Lin G, Zhang R, Yang J, et al. Use of electroacupuncture and transcutaneous electrical acupoint stimulation in reproductive medicine: a group consensus. *J Zhejiang Univ Sci B* 2017;18:186-193.
- Siterman S, Eltes F, Schechter L, Maimon Y, Lederman H, Bartoov B. Success of acupuncture treatment in patients with initially low sperm output is associated with a decrease in scrotal skin temperature. *Asian J Androl* 2009;11:200-208.
- Dieterle S, Li C, Greb R, Bartzsch F, Hatzmann W, Huang D. A prospective randomized placebo-controlled study of the effect of acupuncture in infertile patients with severe oligoasthenozoospermia. *Fertil Steril* 2009;92:1340-1343.
- Zhang C, Zhang CY, Li BB, Zhang HF, Yuan T, Fu YM. Multiglycosides of *Tripterygium wilfordii* increase sperm apoptosis in male rats. *Natl J Androl (Chin)* 2010;16:786-789.
- Li ZR, Fang JQ, Yi SX, eds. *Experimental acupuncture science*. 2th ed. Beijing: Chinese Medicine Press;2007:228-233.
- Zhu WF, ed. *Chinese medicine diagnostic science*. 5th ed. Beijing: Chinese Medicine Press; 2005:195-196.
- Dkhil MA, Zrieq R, Al-Quraishy S, Abdel Moneim AE. Selenium nanoparticles attenuate oxidative stress and testicular damage in streptozotocin-induced diabetic rats. *Molecules* 2016;21:E1517.
- Ma B, Qi H, Li J, Xu H, Chi B, Zhu J, et al. Triptolide disrupts fatty acids and peroxisome proliferator-activated receptor (PPAR) levels in male mice testes followed by testicular injury: a GC-MS based metabolomics study. *Toxicology* 2015;336:84-95.
- Gao J, Zuo Y, So KH, Yeung WS, Ng EH, Lee KF. Electroacupuncture enhances spermatogenesis in rats after scrotal heat treatment. *Spermatogenesis* 2012;2:53-62.
- Zhou L, Xia Y, Ma X, Tang L, Lu J, Tang Q, et al. Effects of "menstrual cycle-based acupuncture therapy" on IVF-ET in patients with decline in ovarian reserve. *Chin Acupunct Moxibust (Chin)* 2016;36:25-28.
- Song FJ, Zheng SL, Ma DZ. Clinical observation on acupuncture for treatment of infertility of ovulatory disturbance. *Chin Acupunct Moxibust (Chin)* 2008;28:21-23.
- Fu B, Lun X, Gong Y. Effects of the combined therapy of acupuncture with herbal drugs on male immune infertility—a clinical report of 50 cases. *J Tradit Chin Med* 2005;25:186-189.
- Huang DM, Huang GY, Lu FE, Stefan D, Andreas N, Robert G. Acupuncture for infertility: is it an effective therapy? *Chin J Integr Med* 2011;17:386-395.
- Zhang M, Huang G, Lu F, Paulus WE, Sterzik K. Influence of acupuncture on idiopathic male infertility in assisted reproductive technology. *J Huazhong Univ Sci Technol Med Sci* 2002;22:228-230.
- Fischl F, Riegler R, Bieglmayer C, Nasr F, Neumark J. Modification of semen quality by acupuncture in subfertile males. *Geburtshilfe Frauenheilkd* 1984;44:510-512.
- Osmanagic-Myers S, Rus S, Wolfram M, Brunner D, Goldmann WH, Bonakdar N, et al. Plectin reinforces vascular integrity by mediating crosstalk between the vimentin and the actin networks. *J Cell Sci* 2015;128:4138-4150.
- Lie PP, Mruk DD, Lee WM, Cheng CY. Cytoskeletal dynamics and spermatogenesis. *Philos Trans R Soc Lond B Biol Sci* 2010;365:1581-1592.
- Dráber P, Sulimenko V, Dráberová E. Cytoskeleton in mast cell signaling. *Front Immunol* 2012;3:130.
- Wloga D, Gaertig J. Post-translational modifications of microtubules. *J Cell Sci* 2010;123:3447-3455.
- Langevin HM, Bouffard NA, Badger GJ, Churchill DL, Howe AK. Subcutaneous tissue fibroblast cytoskeletal remodeling induced by acupuncture: evidence for a mechanotransduction-based mechanism. *J Cell Physiol* 2006;207:767-774.
- Tian HM, Yan J, Yi SX, Zhang YJ, Wen Y. Observation on changes of the intragastric protein phosphorylation level involving electroacupuncture-induced improvement of the injured gastric mucosa in the rat. *Acupunct Res (Chin)* 2009;34:147-151.

(Accepted February 26, 2018; First Online December 28, 2018)  
Edited by WANG Wei-xia