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Effects and mechanism of action of transcutaneous electrical acupuncture point stimulation in patients with abnormal semen parameters

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Abstract

Objective: To evaluate the effect of transcutaneous electrical acupuncture point stimulation (TEAS) on sperm parameters and the underlying molecular mechanisms.

Methods: A total of 121 patients diagnosed with oligozoospermia, asthenozoospermia or oligoasthenozoospermia were randomised into four groups (three treatment groups, one control): the TEAS groups were treated with 2 Hz (n=31), 100 Hz (n=31), or mock stimulation (n=29) at acupuncture points BL23, ST36, CV1 and CV4 for 2 months. The control group (n=30) was provided with lifestyle advice only.

Results: The changes in total sperm count and motility in the 2 Hz TEAS group were significantly greater than those in the mock group and the control group. The change in neutral α-glucosidase (NAG) and zinc levels in the 2 Hz group were significantly greater than those in the mock group and control group, and the changes in fructose levels of the 2 Hz group were significantly greater than those in the control group. Significant increases in calcium and integrin-binding protein 1 (CIB1) and reduction of cyclin-dependent kinase 1 b (CDK1) were also found after 2 Hz TEAS treatment.

Conclusions: The present findings suggest that 2 Hz TEAS can improve sperm count and motility in patients with abnormal semen parameters, and is associated with increases in seminal plasma zinc, NAG and fructose. The upregulation of CIB1 and downregulation of CDK1 by TEAS may be associated with its positive effects on sperm motility and count.

Trial registration: http://www.chictr.org; registration no. ChiCTR-TRC-11001775.

Keywords

transcutaneous electrical acupuncture stimulation, male factor infertility, zinc, calcium and integrin-binding protein 1, cyclin-dependent kinase 1 b

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Introduction

Acupuncture is a part of traditional Chinese medicine that has been used to treat various disorders in the East for more than 3000 years and is now gaining widespread acceptance as a complementary medical treatment. In addition to traditional manual acupuncture (MA), in recent decades new acupuncture modalities such as electroacupuncture (EA)[1,2] and transcutaneous electrical acupuncture point stimulation (TEAS)[3] have gained in popularity.

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About 15% of couples suffer infertility, and approximately one-half of all cases include a male factor.[4] The number of infertile couples appears to be increasing over time.[5] Although a positive effect of MA and EA on sperm parameters has been documented in several studies,[5–7] there is a lack of data suggesting that TEAS has similar effects on sperm parameters.

Seminal plasma contains many cytokines secreted from specific organs or compartments of the male genital system; changes in certain factors, including neutral α-glucosidase (NAG), zinc, fructose and others, have been reported to be associated with semen parameters and used as diagnostic indicators to evaluate the male reproductive system.

In our recent report, poor sperm parameters in humans occurred in concert with changes in calcium and integrin binding protein 1 (CIB1) along with the cell cycle regulator cyclin-dependent kinase 1 (CDK1).[8] Dysregulation of CDK1 or other cell cycle regulators could disrupt the normal interval of Sertoli cell proliferation, causing an imbalance between the number of Sertoli cells and the developing germ cells that can lead to increased germ cell apoptosis and defective spermatogenesis.[9] Thus, alterations in the CIB1 and CDK1 signalling pathway in sperm may be important for the regulation of sperm parameters in patients with oligoasthenozoospermia.

The aim of the present study was to investigate the effects of TEAS in patients with abnormal semen parameters and to test our hypothesis that TEAS influences semen parameters secondary to its effects on biochemical parameters and CIB1 and CDK1 expression.

Methods

Ethics approval

This study was approved by the Ethics Committee of the Second Hospital affiliated to the Shandong University of Traditional Chinese Medicine, China. Each patient gave written informed consent before entering the study and understood that he was free to withdraw from the study at any time. The study was prospectively registered in the Chinese Clinical Trial Registry (ref. ChiCTR-TRC-11001775) on 2 December 2011.

Patients

The study population had been referred to the Reproductive Andrology Laboratory of the Second Hospital affiliated to the Shandong University of Traditional Chinese Medicine by their primary physician due to abnormal findings on semen analysis, between January 2013 and June 2014. A questionnaire was used to collect information from the subjects, including age, height, weight, lifestyle, occupation and environmental exposures, genetic risk factors, sexual and reproductive status, medical history, and physical activity. Men who had ejaculatory dysfunction, a medical history of risk factors for infertility (eg, prior vasectomy, orchidopexy, or varicocele), immune-related infertility, a history of previous infertility treatment (eg, hormonal therapy), documented infection, genetic disease, occupational exposure to agents suspected to impact male reproduction, or who did not return to the centre after TEAS treatment, were excluded from the study.

Diagnostic criteria for oligozoospermia, asthenozoospermia and oligoasthenozoospermia were based on WHO reference values.[10] The diagnosis was confirmed by at least two consecutive semen analyses performed on ejaculates collected after 3–7 days of sexual abstinence.

Transcutaneous electrical acupuncture point stimulation

Patients were assigned to four groups using a computerised randomisation method. Two groups were given TEAS at 2 Hz (n=31) or 100 Hz (n=31), respectively. The third group was given mock TEAS (n=29) with a barely detectable current (5 mA) for 3 s, which was then switched off for 7 s, and served as the placebo control group. The fourth group (n=30) was given lifestyle advice without TEAS to serve as a blank control group. Traditional acupuncture points BL23 (*Shenshu*), ST36 (*Zusanli*), CV1 (*Huiyin*) and CV4 (*Guanyuan*) were chosen based on their use in prior studies on the effect of acupuncture on sperm parameters.[6–11] The stimulation intensity administered to each subject was adjusted according to self-imposed limitations. Patients were trained to perform TEAS on themselves. The TEAS treatment protocol consisted of 30 min daily treatment for 30 days, followed by a rest of 1 to 2 days and then another treatment for 30 days.

Outcome measures

Semen analysis was performed using a computer-assisted semen analysis system (CASA, WLJY-9000; Weili New Century Science and Tech Dev, Beijing, China) according to WHO criteria (2010) for semen volume, sperm concentration, progressive motility and sperm motility).

The semen samples were centrifuged for 20 min at 300 *g*, and the supernatant seminal plasma was quickly and carefully decanted and stored at −20°C until analysis. Seminal plasma levels of zinc, fructose and neutral α-glucosidase (NAG) activity were detected using a ChemWell 2910 autoanalyser (BRED Technology, Shenzhen, China) according to the manufacturer's protocol.

Quantitative real-time reverse transcription PCR

Sperm collection, purification, RNA isolation and cDNA synthesis and quantitative real-time reverse transcription PCR (RT-PCR) were performed as described by Sun et al.[8]

Table 1. Baseline clinical and seminal features of the infertile patients*.

Variable	2 Hz (n=31)	100 Hz (n=31)	Mock TEAS (n=29)	Blank control (n=30)	Statistics
Age, years	31.45 (0.84)	30.94 (1.07)	29.52 (0.81)	31.70 (0.98)	NS
BMI, kg/m^2	24.68 (2.41)	24.23 (2.27)	24.71 (3.78)	25.01 (3.10)	NS
Sexual abstinence time, days	4.65 (0.26)	4.85 (0.36)	5.02 (0.41)	5.37 (0.32)	NS
Semen total volume, mL	2.85 (0.15)	3.14 (0.18)	3.17 (0.16)	3.24 (0.24)	NS
Sperm concentration, $\times 10^6$/mL	20.19 (3.71)	17.44 (3.66)	21.11 (4.17)	17.66 (4.22)	NS
Total sperm count, 10^6/ejaculate	54.69 (9.62)	50.86 (10.96)	65.25 (13.66)	71.17 (24.87)	NS
Progressive motility, %	19.69 (1.77)	22.28 (1.87)	20.52 (1.19)	22.94 (1.69)	NS
Sperm motility, %	32.89 (2.23)	35.10 (2.40)	37.41 (2.47)	36.42 (1.66)	NS

*Data are presented as mean (SEM).
BMI, body mass index; NS, non-significant difference; TEAS, transcutaneous electrical acupuncture point stimulation.

Western blot analysis

All sperm cells were lysed in RIPA lysis buffer (Dingguo, Beijing, China). Protein concentration was determined by the Lowry method with the absorbance read on a Q5000 spectrophotometer (Quwell, San Jose, CA, USA). Western blot analysis was performed as described by Sun et al.[8]

Statistical analysis

The data are presented as mean±SEM. Differences between groups were determined by one-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test. All intragroup comparisons were made using a paired t-test to examine for differences before and after stimulation. All statistical analyses were performed with GraphPad Prism 5 software for Windows (GraphPad Software Inc., San Diego, CA, USA).

Results

Demographic and baseline characteristics of the study population

As shown in table 1, there were no statistically significant differences in age, body mass index (BMI), sexual abstinence time, total semen volume, sperm concentration or sperm motility across groups.

Changes in sperm parameters before and after TEAS treatment

The effect of TEAS treatment on sperm parameters is shown in table 2. TEAS at 2 Hz and 100 Hz significantly increased sperm concentration, progressive motility and sperm motility. No significant differences in sperm concentration or motility were observed after mock TEAS or in the blank control group.

Effect of TEAS treatment on sperm and seminal plasma biochemical parameters

Changes in sperm parameters are shown in figure 1. The change in total sperm count was significantly higher in the 2 Hz group (47.5±9.7%) compared with the mock TEAS (7.9±7.2%, $P<0.01$) and blank control groups (2.0±6.8%, $P<0.01$; Figure 1A). The change in total percentage of progressively motile sperm was significantly higher in the 2 Hz group (40.1±14.0%) compared with the mock TEAS (0.1±3.1%, $P<0.01$) and blank control groups (4.1±2.3%, $P<0.05$; Figure 1B). Furthermore, the change in sperm motility was significantly higher in the 2 Hz group (20.0±5.4%) compared with the mock TEAS (−1.2±3.4%, $P<0.01$) and the blank control groups (2.8±1.8%, $P<0.05$; Figure 1C).

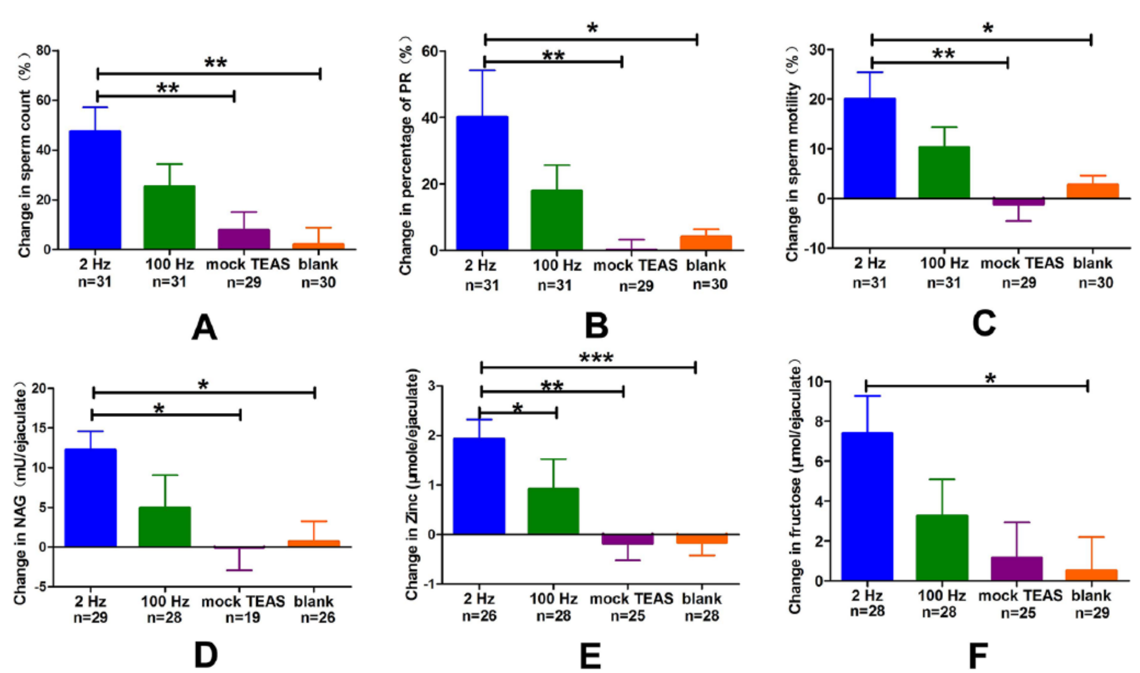
Due to low seminal volume or technical reasons, NAG, zinc and fructose were only measured in 102, 107 and 110 of the 121 semen samples, respectively. As shown in Figure 1D, the change in the total level of NAG was significantly greater in the 2 Hz group (12.95±2.58 mU/ejaculate) when compared with the mock TEAS (−0.06±3.10 mU/ejaculate, $P<0.05$) and blank control groups (0.68±2.58 mU/ejaculate, $P<0.05$). The change in zinc was significantly greater in the 2 Hz group (1.93±0.39 μmol/ejaculate) compared with the 100 Hz group (0.92±0.61 μmol/ejaculate, $P<0.05$), mock TEAS (−0.16±0.37 μmol/ejaculate, $P<0.01$) and blank control groups (−0.16±0.26 μmol/ejaculate, $P<0.001$; Figure 1E). The change in fructose level was significantly greater in the 2 Hz group (7.40±1.88 μmol/ejaculate) compared with the blank control group (0.52±1.68 μmol/ejaculate, $P<0.05$; Figure 1F).

Table 2. Changes in sperm parameters after TEAS treatment†.

Variable	Time	2 Hz TEAS (n=31)	100 Hz TEAS (n=31)	Mock TEAS (n=29)	Blank control (n=30)
Sexual abstinence time, days	Before	4.57 (0.26)	4.86 (0.36)	5.02 (0.41)	5.37 (0.32)
	After	4.40 (0.21)	4.82 (0.32)	5.37 (0.32)	5.27 (0.36)
Sperm concentration, $\times 10^6$/mL	Before	20.19 (3.71)	17.44 (3.66)	21.11 (4.17)	17.66 (4.22)
	After	24.07 (3.64)**	18.93 (3.58)*	21.20 (3.73)	16.66 (3.85)
Total sperm count, 10^6/ejaculate	Before	54.69 (9.62)	50.86 (10.96)	65.25 (13.66)	71.17 (24.88)
	After	73.09 (11.43)**	61.18 (13.56)	69.12 (14.40)	63.73 (20.23)
Progressive motility, %	Before	19.69 (1.77)	22.28 (1.82)	20.52 (1.19)	22.94 (1.69)
	After	23.21 (1.60)**	24.35 (2.03)*	20.09 (1.06)	24.12 (1.67)*
Sperm motility, %	Before	32.89 (2.23)	35.10 (2.40)	37.41 (2.47)	36.42 (1.66)
	After	37.78 (2.23)*	37.71 (2.50)*	35.53 (1.71)	37.16 (1.84)

*P<0.05, **P<0.01, all versus before TEAS.
†Data are presented as mean (SEM).
TEAS, transcutaneous electrical acupuncture point stimulation.

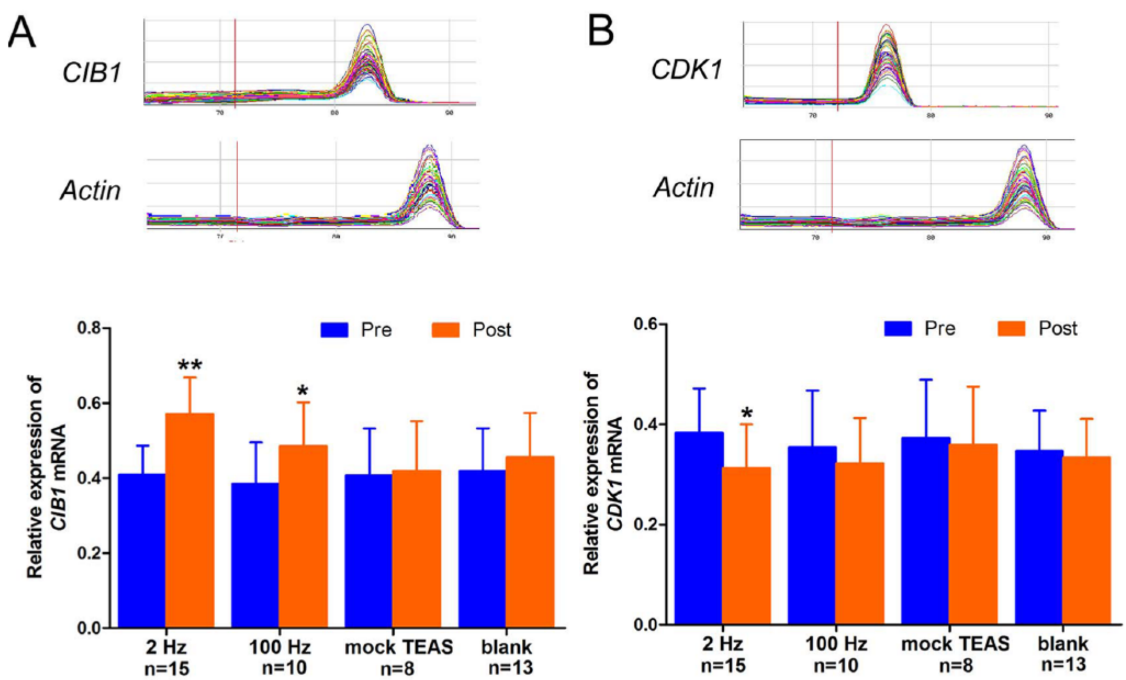
Figure 1. Changes in sperm and seminal plasma biochemical parameters in control and TEAS groups. (A) Sperm count. (B) Percentage of progressive motility (PR). (C) Sperm motility. (D) NAG. (E) Zinc. (F) Fructose. *P<0.05; **P<0.01, ***P<0.001, one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test. NAG, neutral α-glucosidase; TEAS, transcutaneous electrical acupuncture point stimulation.



Effects of TEAS treatment on CIB1 and CDK1 mRNA expression

As shown in Figure 2A, paired t-tests indicated that *CIB1* gene expression was significantly enhanced after TEAS treatment within the 2 Hz group (0.41±0.08 vs 0.57±0.10, P<0.01) and 100 Hz group (0.38±0.11 vs 0.49±0.12, P<0.05). By contrast, *CDK1* gene expression was significantly decreased after TEAS treatment

Figure 2. Comparisons of *CIB1* and *CDK1* mRNA expression in sperm pre- and post- TEAS treatment or lifestyle advice. (A) *CIB1*. (B) *CDK1*. Upper panel: representative melting curve. Lower panel: summary of relative *CIB1* and *CDK1* mRNA expression normalised to the internal control, *Actin*. 2 Hz TEAS induced a statistically significant upregulation of *CIB1* and downregulation of *CDK1* mRNA expression in sperm. *P<0.05; **P<0.01, one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test. *CDK1*, cyclin-dependent kinase 1 b; CIB1, calcium and integrin-binding protein 1; TEAS, transcutaneous electrical acupuncture point stimulation.



in the 2 Hz group (0.38±0.09 vs 0.31±0.09, P<0.05; Figure 2B).

Effects of TEAS treatment on CIB1 and CDK1 protein expression

As shown in Figure 3, 2 Hz TEAS induced a prominent increase in CIB1 protein expression (0.69±0.07 vs 1.30±0.11, P<0.05), as well as a statistically significant reduction in CDK1 protein expression (3.54±0.43 vs 2.15±0.22, P<0.01). Protein expression of CIB1 and CDK1 did not change significantly in the 100 Hz group.

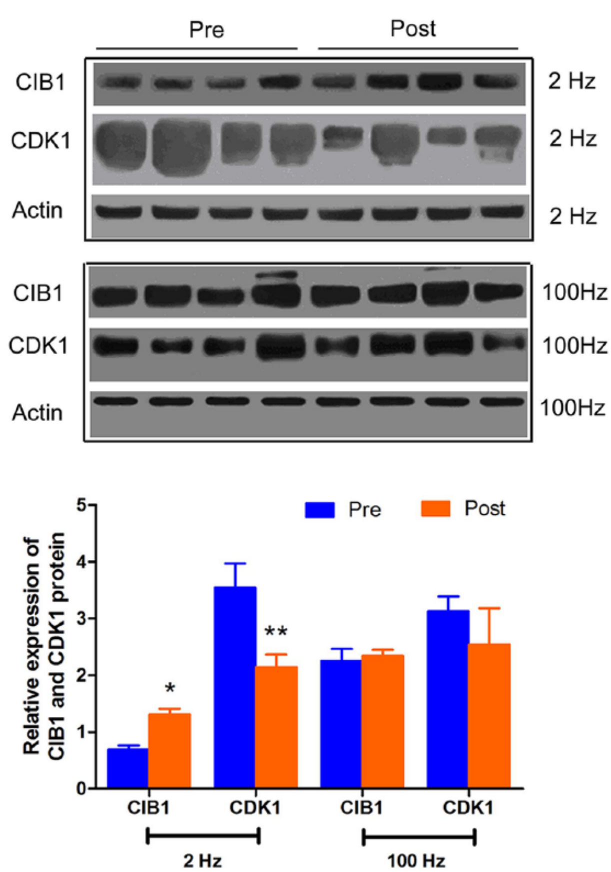
Discussion

A portable TEAS device, named the Han's Acupuncture point Nerve Stimulator (HANS), was introduced to clinical practice in the late 1980s. Unlike MA, which uses manual needling to achieve a therapeutic effect, TEAS delivers electrical pulses to the tissues located at the sites of traditional acupuncture points via self-adhesive electrodes, placed on the surface of the skin, with the added advantage of increased reproducibility, and great savings in manpower. Recently it has been reported that TEAS significantly improved the clinical outcome of embryo transfer in women.[12,13] In this study, we have provided experimental evidence that TEAS can be effective in the case of oligoasthenozoospermia.

A previous investigation reported significant increases in the percentages of rapidly motile and morphologically normal sperm after acupuncture intervention.[14] Dieterle et al.[6] also demonstrated a significant effect of acupuncture on the percentage of total motile sperm, while Gao et al.[7] found that EA enhances germ cell proliferation through improvement of Sertoli cell function in rats, and may enhance spermatogenesis and restore normal semen parameters in subfertile patients. Our study demonstrated that TEAS improved the sperm count and motility of patients with abnormal semen parameters. By contrast, a systematic review of the role of acupuncture in male subfertility found no sufficient evidence to support such a role.[15]

Sham acupuncture, in which acupuncture needles are inserted at locations not corresponding to traditional acupuncture points, has been criticised as a control because physiological effects from needle insertion *per se* are likely to be present. Placebo TEAS is arguably a superior control as it can be performed with no, or very weak, stimulation. Accordingly, in the present study, mock TEAS was used as

Figure 3. Comparisons of CIB1 and CDK1 protein expression in sperm pre- and post- 2 Hz and 100 Hz TEAS treatment. Upper panel: representative Western blotting bands. Lower panel: analysis of the relative intensity of CIB1 and CDK1 protein. Actin was used as an internal control. $^*P<0.05$; $^{**}P<0.01$, one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test, n=8 patients per group. CDK1, cyclin-dependent kinase 1 b; CIB1, calcium and integrin-binding protein 1; TEAS, transcutaneous electrical acupuncture point stimulation.



a sham intervention in order to control for possible effects of psychological and other non-physiological factors on parameters of male infertility.

Several biochemical markers of epididymal function can be found in seminal fluid, with the activity of NAG considered one of the most useful biomarkers from a clinical perspective.[16] A number of studies have shown positive correlations between seminal levels of NAG and sperm motility.[17] An optimal level of NAG may provide appropriate energy for sperm maturation. It has been reported that sperm with higher α-glucosidase activity have a greater ability to bind to the zona pellucida.[18] Zinc plays an important role in sperm function, with reports indicating a positive correlation between seminal zinc levels and sperm motility and concentration.[19] Fructose has been reported as a source of energy for gamete motility. Seminal levels of fructose have been reported to demonstrate a positive correlation with sperm motility and sperm count.[17-20] We found that sperm count, sperm motility, and seminal levels of NAG, zinc and fructose were increased after TEAS treatment, especially in the 2 Hz group. These results suggest that sperm count and sperm motility may be significantly improved via the increases in seminal NAG, zinc and fructose levels after 2 Hz TEAS treatment. These results add to our understanding of the impact of biochemical markers on sperm count and sperm motility.

EA has been shown to increase uterine blood flow and modulate reproductive hormone levels in infertile women.[21,22] EA has also been reported to increase testicular blood flow[23] and improve reproductive endocrine function.[2] The hypothalamic β-endorphin system is known to play a regulatory role in a variety of functions, including the reproductive and autonomic nervous systems.[24] β-endorphin can induce a general decrease in sympathetic tone, manifested by a reduction in blood pressure and decreased muscle sympathetic nerve activity.[25] The autonomic nervous system has also been shown to influence vasoactivity within the testis. 2 Hz TEAS has been shown to induce release of β-endorphin specifically,[26] which may represent another mechanism.

Several studies have demonstrated that calcium (Ca^{2+}) signalling serves as a central regulator in many key activities of sperm cells, including capacitation, hyperactivation, chemotaxis and the acrosome reaction.[27] It is well established that control of motility, including hyperactivation and chemotaxis, is particularly dependent on intracellular free Ca^{2+} signalling in the principal piece of the flagellum and the midpiece.[28] Repression of the CIB1 channel in sperm cells impairs Ca^{2+} signalling, which in turn causes impairment of sperm motility and hyperactivation associated with asthenozoospermia and male subfertility.[29] In support of this notion, male mice deficient in the *CIB1* gene demonstrate impairments in both sperm motility and count and are completely infertile.[30] Consistent with these findings, in a previous report we observed a significant reduction in the level of *CIB1* gene expression in patients with oligoasthenozoospermia.[8] In addition, *CIB1* expression in sheep is highest in the testis.[31] CIB1 mRNA and protein levels in the sperm were significantly higher after 2 Hz TEAS treatment compared with the other groups. Thus, it is possible that the observed effect of TEAS treatment on sperm parameters in patients with abnormal semen parameters was associated with the regulation of CIB1 in sperm. Together with our previous findings, our results suggest that repression of CIB1 channels in sperm may contribute to abnormal semen parameters, while the upregulation of CIB1 expression and downregulation of CDK1 expression after TEAS appears to be involved in the effects of TEAS on subfertility.

In our previous study, CDK1 protein levels increased in patients with oligoasthenozoospermia and asthenozoospermia, with the highest level reached in oligoasthenozoospermic patients. Changes in CDK1 expression can disrupt the normal interval of Sertoli cell proliferation, causing an imbalance between the numbers of Sertoli cells and developing germ cells, which may lead to increased germ cell apoptosis and defective spermatogenesis.[9] Sertoli cells secrete a variety of endocrine and paracrine factors that regulate spermatogenesis.[32] In the present study, the observed upregulation of CIB1 may reflect a compensatory increase that is directly related to the loss of CDK1 after TEAS treatment in these patients.

It was found that the frequency of EA stimulation is one of the key factors in determining its therapeutic efficacy.[33,34] For example, the increase in gastric motility amplitude in rats induced by MA at 2 Hz was greater than for all other frequencies,[33] whereas in spinal cord injury-induced muscle spasticity only 100 Hz, and not 2 Hz, produced a spasmolytic effect.[34] This effect was shown to be frequency-dependent.

Our study has some limitations. First, since only low-frequency (2 Hz) and high-frequency (100 Hz) TEAS were used to treat patients with abnormal semen parameters, it is possible that a mid-range frequency (such as 15 Hz) or low and high alternating frequency (DD mode) may be more effective for improving sperm parameters. Another key limiting factor was the fact we did not test the mRNA and protein expression levels of CIB1 and CDK1 in testicular and epididymal spermatozoa. Further investigations in this area are required.

Conclusion

Our results indicate that TEAS may be an effective therapeutic strategy for the treatment of male infertility. TEAS can effectively improve semen parameters, biochemical markers and the dysregulation of CIB1 and CDK1 expression in the sperm of patients with abnormal semen analysis. These findings support our hypothesis that TEAS exerts its positive effect on abnormal semen parameters by upregulating biochemical regulators and the CIB1 channel and downregulating the CDK1 channel in sperm.

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Contributors

YY designed the study, performed the data analysis and drafted the manuscript. WS designed the study and participated in the discussion of the results. JW revised the data analysis and the manuscript. SS and BZ trained patients in the use of the HANS device, collected the semen samples and patient information, and carried out the semen analysis. QG and ML measured the seminal plasma biochemical markers and participated in relevant discussions. LZ and QZ carried out the qRT-PCR and Western blotting. All authors read and approved the final version of the manuscript accepted for publication.

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Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Patient consent

Obtained.

Ethics approval

This study was approved by the Ethics Committee of the Second Hospital affiliated to the Shandong University of Traditional Chinese Medicine.

Provenance and peer review

Not commissioned; externally peer reviewed.

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