

ORIGINAL RESEARCH ARTICLE

Effect of acupuncture on sperm mitochondrial function: A study based on the Keap1–nuclear factor erythroid 2-related factor 2/heme oxygenase-1 signaling pathway

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Abstract

Introduction: Asthenozoospermia, characterized by impaired sperm motility, is a major contributor to male infertility. Mitochondrial dysfunction driven by oxidative stress plays a critical role in its pathogenesis, and the Kelch-like ECH-associated protein 1 (Keap1)–nuclear factor erythroid 2-related factor 2/heme oxygenase-1 (Nrf2/HO-1) signaling pathway serves as the principal regulator of cellular antioxidant defense. Acupuncture has shown clinical benefits for sperm quality, yet its underlying molecular mechanism remains inadequately defined.

Objective: To examine the regulating influence of acupuncture on mitochondrial oxidative stress in asthenozoospermic rat sperm and to clarify its underlying mechanism related to the Keap1–Nrf2/HO-1 signaling pathway.

Methods: Thirty-two male Sprague–Dawley rats were randomly assigned to four groups ($n=8$): normal, model, acupuncture, and curcumin. After model induction with ornidazole, the acupuncture group received electro-acupuncture (2 Hz, 2 mA) at Guanyuan (CV4), Shenshu (BL23), Zusanli (ST36), and Sanyinjiao (SP6) for 28 days, while the curcumin group received oral curcumin (300 mg/kg/day). Sperm motility was assessed using computer-assisted sperm analysis. Mitochondrial membrane potential (MMP) was measured using JC-1 staining. Oxidative stress markers were evaluated by biochemical assays. *Keap1*, *Nrf2*, and *HO-1* mRNA and protein expression were determined by real-time quantitative polymerase chain reaction and Western blotting.

Results: Compared with the normal group, the model group exhibited significantly reduced sperm motility and MMP, elevated malondialdehyde levels, and decreased superoxide dismutase and glutathione peroxidase activities ($p<0.01$). Both acupuncture and curcumin effectively reversed these changes ($p<0.01$). Furthermore, acupuncture downregulated Keap1 expression while upregulating Nrf2 and HO-1 at both transcriptional and translational levels ($p<0.01$), consistent with the curcumin group.

Conclusion: Acupuncture improves sperm motility in ornidazole-induced asthenozoospermic rats by stimulating the Keap1–Nrf2/HO-1 signaling pathway,

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hence reducing oxidative stress, maintaining mitochondrial function, and increasing antioxidant capacity. These findings provide mechanistic evidence for acupuncture as a viable treatment for oxidative stress-related male infertility.

Keywords: Acupuncture; Asthenozoospermia; Sperm mitochondria; Oxidative stress; Keap1–nuclear factor erythroid 2-related factor 2/heme oxygenase-1

1. Introduction

Infertility, characterized as the inability to conceive after 12 months of consistent unprotected coitus, impacts millions of couples globally.¹ Male infertility constitutes around 30–50% of all cases, with a global prevalence estimated at 56.53 million in 2019, of which China represented 25.79%.^{2,3} In recent years, diminishing sperm characteristics, especially sperm motility, have garnered heightened concern as a significant hazard to human reproductive health.⁴ Asthenozoospermia (AZS) is a condition characterized predominantly by diminished sperm motility, in which the percentage of progressively motile sperm is less than 32% and the sperm concentration exceeds 15 million per mL.⁵ AZS represents a critical etiological factor for male infertility.⁶ Clinically, AZS accounts for approximately 18% of cases in infertility clinics, with some studies reporting rates as high as 59% among men with abnormal semen parameters.^{7,8} The etiology of AZS is multifactorial, encompassing genetic mutations, oxidative stress, varicocele, infections, and lifestyle factors.⁹ Despite its substantial clinical significance, isolated AZS is frequently underrecognized. This distinct subset of male infertility is defined solely by impaired sperm motility, occurring independently of any abnormalities in sperm concentration or morphology.

Spermatozoa are highly specialized cells, and their capacity for progressive motility—an essential prerequisite for natural fertilization—relies critically on a continuous and robust supply of adenosine triphosphate (ATP). This energy demand is predominantly met by mitochondria, which are strategically localized in the midpiece of the sperm flagellum to fuel axonemal dynein ATPase activity and thereby sustain flagellar beating.^{10,11} Any deficiency in mitochondrial bioenergetics directly undermines sperm motility. A robust link has been demonstrated between mitochondrial dysfunction and the development of AZS.^{12–14} The primary indication of mitochondrial functional integrity is the mitochondrial membrane potential (MMP), produced by proton translocation across the inner membrane during electron transport chain

activity. A reduction in MMP indicates compromised oxidative phosphorylation and reduced ATP-generating capacity, ultimately resulting in lower sperm motility.^{13,14}

Mitochondrial dysfunction is significantly affected by oxidative stress, a state defined by an imbalance between the production of reactive oxygen species (ROS) and the efficacy of intrinsic antioxidant defenses.¹⁵ Physiological levels of ROS are essential for optimal mitochondrial activity; however, excessive ROS causes structural and functional impairment to sperm mitochondria, resulting in decreased ATP production, reduced motility, and potential DNA damage.^{5,16,17} Oxidative stress is recognized as a major contributor to the pathogenesis of AZS.^{18,19} Thus, preserving mitochondrial integrity and enhancing antioxidant capacity generally represent promising therapeutic strategies for mitigating ROS-associated AZS.

The Kelch-like ECH-associated protein 1 (Keap1)–nuclear factor erythroid 2-related factor 2/heme oxygenase-1 (Nrf2/HO-1) signaling pathway functions as the principal regulator of cellular antioxidant defense.^{20,21} ROS modify critical cysteine residues on Keap1 during oxidative stress, leading to a conformational change. This conformational alteration inhibits Nrf2 ubiquitination, thereby facilitating its nuclear translocation. Nrf2 associates with antioxidant response elements in the nucleus, augmenting the synthesis of cytoprotective enzymes such as HO-1, which mitigates oxidative damage.²²

Conventional therapeutic strategies for AZS, including lifestyle modifications, varicocele repair, and empirical antioxidant supplementation, have yielded inconsistent and often suboptimal clinical outcomes. Although antioxidant therapy has demonstrated potential to improve semen parameters, its success remains limited, and a clear consensus regarding the optimal types, dosages, and treatment durations is lacking. Moreover, several studies have failed to demonstrate any positive effect of antioxidant administration, with some even reporting detrimental impacts on male fertility. This therapeutic uncertainty underscores an urgent need for novel, mechanism-targeted interventions that directly address the molecular pathways

governing redox homeostasis in the male reproductive tract.

Acupuncture, a fundamental component of traditional Chinese medicine, has demonstrated therapeutic efficacy in improving sperm parameters in both clinical and experimental studies.^{23–25} Recent research indicates that acupuncture may have antioxidant benefits, possibly by modulating the Keap1–Nrf2 signaling pathway.^{26,27} Nonetheless, the exact molecular pathways by which acupuncture enhances sperm mitochondrial activity remain inadequately elucidated.

This study investigates the impact of acupuncture on sperm motility in a rat model of ornidazole-induced AZS by activating the Keap1–Nrf2/HO-1 signaling pathway, thereby mitigating oxidative stress and restoring mitochondrial function. Our research provides a mechanistic insight into the therapeutic efficacy of acupuncture for male infertility linked to oxidative stress.

2. Materials and methods

2.1. Animals and ethical approval

Thirty-two male Sprague–Dawley rats (4–5 weeks old, 230 ± 30 g, SPF grade; Beijing HuaFuKang Biological Technology Co., Ltd., License No. SCXK (Jing) 2024-0003) were housed under controlled environmental parameters (21 ± 1 °C, $55 \pm 5\%$ relative humidity, 12/12 h light-dark cycle) with *ad libitum* access to standard chow and water. The Animal Ethics Committee of Yishengyuan Gene Technology (Tianjin) Co., Ltd. approved all procedures (Approval No. YSY-DWLL-2023601).

2.2. Reagents and instruments

Ornidazole and curcumin were sourced from Shanghai Macklin Biochemical Technology Co., Ltd. (China). Disposable sterile acupuncture needles (0.25×40 mm) were purchased from Suzhou Medical Supplies Co., Ltd. (China). Electroacupuncture stimulation was delivered using a Hwato (China) electronic acupuncture apparatus.

2.3. Model establishment and intervention protocol

Following a week of acclimatization, 32 rats were randomly assigned to four groups ($n = 8$ per group): normal group (NG), model group (MG), acupuncture group (AG), and curcumin group (CG) via a stratified randomization method based on body weight to assure baseline uniformity. Rats were categorized by body weight and organized into blocks of four, with each rat within a block randomly assigned to one of the four groups using a computer-generated random number sequence (Microsoft Excel RAND function). Rats in the NG were administered

a 1% carboxymethylcellulose sodium (CMC-Na) solution via gavage for 56 days. The MG, AG, and CG received ornidazole solution (400 mg/kg, dissolved in 1% CMC-Na) by gavage for 28 days to develop the AZS model.²⁸ On day 28, two rats from the MG were randomly selected and euthanized to assess sperm quality for model validation. Beginning on day 29, the MG was administered CMC-Na solution via gavage for a duration of 28 days; the AG underwent electro-acupuncture, with rats secured on a rodent frame and needles inserted vertically at Guanyuan (CV4), Shenshu (BL23), Zusanli (ST36), and Sanyinjiao (SP6)²⁹ in accordance with standardized rat acupoint locations (Figure A1). Needles were attached to an electro-acupuncture apparatus (2 Hz, 2 mA, 30 min/day); the control group received a daily gavage of curcumin solution (300 mg/kg, dissolved in CMC-Na). Both treatments lasted 28 days. All groups were provided with uniform food, water, and lodging.

The explicit nature of the acupuncture intervention rendered it impractical to blind the staff giving the therapy. Nonetheless, outcome assessors were rigorously blinded to group assignment. All sperm analyses, histological assessments, flow cytometry, and biochemical tests were performed by laboratory workers blinded to the experimental group allocations. Sample vials and data files were anonymized by an independent technician, with the code revealed only after the completion of all statistical analyses.

2.4. Sample collection

Body weight was measured 24 h after the final intervention. Subsequent to death via cervical dislocation, the epididymides were promptly removed from the rats. The epididymal tail from one side was preserved in 4% paraformaldehyde for 24 h for histological evaluation, while the other side was utilized for sperm analysis. The leftover tissue specimens were stored at -80 °C for further examination.

2.5. Semen analysis

Sperm suspensions were prepared from minced epididymal tails and incubated at 37 °C under 5% CO_2 . After dilution, sperm concentration and progressive motility were evaluated using computer-aided sperm analysis.

2.6. Histological examination

Fixed epididymal tissues underwent standard dehydration, clearing, and paraffin embedding. Sections ($4\text{--}5$ μm) were stained with Harris hematoxylin and eosin (HE) and evaluated microscopically (Olympus BX53, Olympus, Japan).

2.7. Mitochondrial membrane potential assessment

Mitochondrial membrane potential was measured using a JC-1 kit (Servicebio, China) according to the manufacturer's protocol. Sperm collected from the cauda epididymis were incubated with JC-1 working solution at 37 °C for 20 min in the dark. After two washes, fluorescence was recorded by flow cytometry (BD FACSCanto II, BD Biosciences, United States).

2.8. Oxidative stress markers

Epididymal homogenates were prepared in ice-cold phosphate-buffered saline and clarified by centrifugation (12,000× g, 10 min, 4 °C). Total protein concentration was quantified using the bicinchoninic acid assay (BIOSHARP, China) with a bovine serum albumin standard curve, followed by normalization of enzyme activity. Malondialdehyde (MDA) content was measured using the thiobarbituric acid (TBA) assay (Nanjing Jiancheng Bioengineering Institute, China), with absorbance measured at 532 nm. Superoxide dismutase (SOD) activity was assessed using the WST-8 method (Nanjing Jiancheng Bioengineering Institute, China) with absorbance detection at 450 nm. Glutathione peroxidase (GSH-Px) activity was determined using a colorimetric assay (Nanjing Jiancheng Bioengineering Institute, China) with absorbance detection at 412 nm. Enzyme activities were expressed as U/mg protein.

2.9. RNA extraction and quantitative real-time polymerase chain reaction

Total RNA was isolated with TRIzol reagent. Reverse transcription used 1 µg RNA and a commercial cDNA synthesis kit. Quantitative polymerase chain reaction (PCR) was performed on a QuantStudio 1 system (ThermoFisher Scientific, United States) using SYBR Green premix. Primer sequences for *Keap1*, *Nrf2*, *HO-1*, and 18S rRNA are listed in Table 1. Amplification conditions: initial

denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 62 °C for 30 s. Relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method with normalization to 18S rRNA.

2.10. Western blot

Tissue lysates were prepared in radioimmunoprecipitation assay buffer containing protease inhibitors, and protein concentrations were determined by bicinchoninic acid assay. Samples (20–30 µg protein/lane) were resolved on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels and transferred to polyvinylidene fluoride membranes. After blocking in 5% non-fat milk/Tris-buffered saline with Tween 20 (2 h, room temperature), membranes were probed overnight at 4 °C with primary antibodies: anti-Keap1 (1:1,000; 60027-1-IG, Proteintech, United States), anti-Nrf2 (1:1,000; 16396-1-AP, Proteintech, United States), anti-HO-1 (1:1,000; ab189491, Abcam, United Kingdom), and anti-β-tubulin (loading control). Following Tris-buffered saline with Tween 20 washes, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (1:5,000; ZB-2305 [anti-mouse], ZB-2301 [anti-rabbit]; ZSGB-BIO, China) for one hour at room temperature. Signals were visualized with enhanced chemiluminescence reagent and an Amersham Imager 600 system (GE Healthcare Life Sciences, United States). Densitometry was performed using ImageJ (version 1.54), and target protein levels were normalized to β-tubulin.

2.11. Statistical analysis

Data are presented as mean ± standard deviation. Normality and homogeneity of variance were assessed by Shapiro-Wilk and Levene's tests, respectively. One-way analysis of variance followed by Tukey's post hoc test was used for multiple comparisons. A *p*-value < 0.05 was considered statistically significant. Analyses were conducted with GraphPad Prism 10.

Table 1. Primer sequences

Gene	Primer sequences	Product length (bp)
<i>Keap1</i> forward	5'- TGCTCAACCGCTTGCTGTATG-3'	100
<i>Keap1</i> reverse	5'- ATCATCCGCCACTCATTCCTCT-3'	100
<i>Nrf2</i> forward	5'- CCTTCCTCTGCTGCCATTAGTC -3'	111
<i>Nrf2</i> reverse	5'- CGTGCCTTCAGTGTGCTTCT - 3'	111
<i>HO-1</i> forward	5'- CGCATGAACACTCTGGAGATGA -3'	144
<i>HO-1</i> reverse	5'- CTGTGAGGGACTCTGGTCTTTG -3'	144
18s rRNA forward	5'-GACACGGACAGGATTGACAGAT-3'	122
18s rRNA reverse	5'-TGCCAGAGTCTCGTTTCGTTATC-3'	122

3. Results

3.1. General and semen parameters

No statistically significant difference in body weight was observed among the four groups ($p > 0.05$). Conversely, the MG exhibited substantial reductions in epididymal weight, sperm progressive motility, and sperm concentration relative to the NG ($p < 0.05$ or $p < 0.01$). Following 28 days of intervention, both acupuncture and curcumin therapy dramatically elevated these parameters to near-normal levels ($p < 0.01$). Comprehensive numerical statistics are provided in Table 2.

3.2. Histopathological examination

Hematoxylin and eosin staining revealed well-organized epididymal tubules with abundant sperm in the NG. The MG exhibited disrupted tubular architecture, vacuolization, and disorganized cilia. Acupuncture and curcumin treatments ameliorated these histopathological changes, resulting in more compact tubular structures and reduced vacuolization (Figure A2).

3.3. Mitochondrial membrane potential

Table 3 indicates that the mean MMP value in the MG (27.41%) was substantially lower than that in the NG (51.16%, $p < 0.01$). Acupuncture therapy increased MMP to 53.72% and curcumin to 50.69%, both considerably beyond the MG ($p < 0.01$). No notable difference was observed between the AG and CG.

3.4. Oxidative stress markers

Figure 1 demonstrates that the MG exhibited a substantial elevation in MDA concentration, along with significant reductions in SOD and GSH-Px activity relative to the NG ($p < 0.01$). Both acupuncture and curcumin therapy markedly reduced MDA levels and enhanced SOD activity ($p < 0.01$). Acupuncture significantly elevated GSH-Px activity ($p < 0.05$), whereas curcumin exhibited no statistically significant impact on this measure ($p > 0.05$).

3.5. mRNA expression levels

Quantitative real-time PCR results (Figure 2) indicated that the MG exhibited significantly elevated Keap1 mRNA expression and reduced Nrf2 and HO-1 mRNA levels compared to the NG ($p < 0.01$). Both acupuncture and curcumin therapy rectified these expression patterns, leading to decreased Keap1 and elevated Nrf2 and HO-1 transcripts ($p < 0.01$ for all comparisons vs the MG).

3.6. Protein expression level

The Western blot analysis (Figure 3) validated the mRNA results. In the MG, the expression of Keap1 protein was increased, whereas the levels of Nrf2 and HO-1 proteins were reduced in comparison to the NG ($p < 0.01$). Both acupuncture and curcumin therapies markedly reinstated these protein expression patterns, resulting in the downregulation of Keap1 and the upregulation of Nrf2 and HO-1 ($p < 0.01$).

Table 2. Comparison of body weight, epididymal weight, and semen parameters

Group	<i>n</i>	Body weight (g)	Epididymal weight (g)	Progressive motility ratio (%)	Sperm count ($\times 10^6/\text{mL}$)
NG	8	424.51 $\pm$ 6.79	1.56 $\pm$ 0.09	27.67 $\pm$ 7.33	32.19 $\pm$ 8.62
MG	8	421.83 $\pm$ 6.41	1.41 $\pm$ 0.11 [*]	12.61 $\pm$ 1.79 ^{**}	18.78 $\pm$ 5.15 [*]
AG	8	426.43 $\pm$ 3.44	1.47 $\pm$ 0.05	23.49 $\pm$ 2.02 [#]	36.63 $\pm$ 10.86 [#]
CG	8	426.13 $\pm$ 3.84	1.49 $\pm$ 0.04	23.91 $\pm$ 5.66 [#]	29.04 $\pm$ 3.25
<i>F</i> -statistics		1.271	4.997	14.46	8.148
<i>p</i>		0.3036	0.0067	<0.01	0.0005

Note: ^{*} $p < 0.05$ (versus NG); ^{**} $p < 0.01$ (versus NG); [#] $p < 0.01$ (versus MG).

Abbreviations: AG: Acupuncture group; CG: Curcumin group; MG: Model group; NG: Normal group.

Table 3. Comparison of sperm mitochondrial membrane potential

Group	NG	MG	AG	CG	<i>F</i>	<i>p</i>
MMP (%)	51.16 $\pm$ 13.11	27.41 $\pm$ 6.04 [*]	53.72 $\pm$ 6.92 [#]	50.69 $\pm$ 4.17 [#]	17.69	<0.01

Note: ^{*} $p < 0.01$ (versus NG); [#] $p < 0.01$ (versus MG).

Abbreviations: AG: Acupuncture group; CG: Curcumin group; MG: Model group; MMP: Mitochondrial membrane potential; NG: Normal group.

4. Discussion

This work reveals, for the first time, that electroacupuncture therapy improves sperm motility in an ornidazole-induced rat model of AZS by altering the Keap1–Nrf2/HO-1 signaling pathway, therefore diminishing oxidative damage to sperm mitochondria. Impaired sperm motility is the defining characteristic of AZS, and growing evidence suggests that mitochondrial dysfunction is a primary pathogenic contributor. Therefore, interventions designed to maintain or restore mitochondrial activity provide therapeutic potential. This work demonstrated that ornidazole treatment effectively replicated essential characteristics of AZS, as indicated by substantial reductions in sperm motility, sperm count, and MMP, hence validating the creation of an oxidative stress-mediated model of sperm destruction. Following the acupuncture session, these motility metrics showed significant recovery, and MMP values returned to levels comparable to those

observed with the positive control drug curcumin. These data substantiate the idea that acupuncture positively influences sperm energy metabolism.

The assessment of oxidative stress indicators showed that the model group exhibited significant lipid peroxidation and reduced activity of the antioxidant enzymes SOD and GSH-Px, indicating an impaired redox equilibrium. Acupuncture therapy reduced MDA levels while simultaneously increasing SOD and GSH-Px activities, underscoring its significant antioxidant efficacy. These findings correspond with previous studies indicating that acupuncture enhances tissue function by restoring redox balance.^{30–32} Notably, acupuncture appears to enhance GSH-Px activity more effectively than curcumin, suggesting that its antioxidant effects may involve broader or complementary regulatory mechanisms beyond those activated by curcumin. This discrepancy is mechanistically plausible but remains speculative. We hypothesize that,

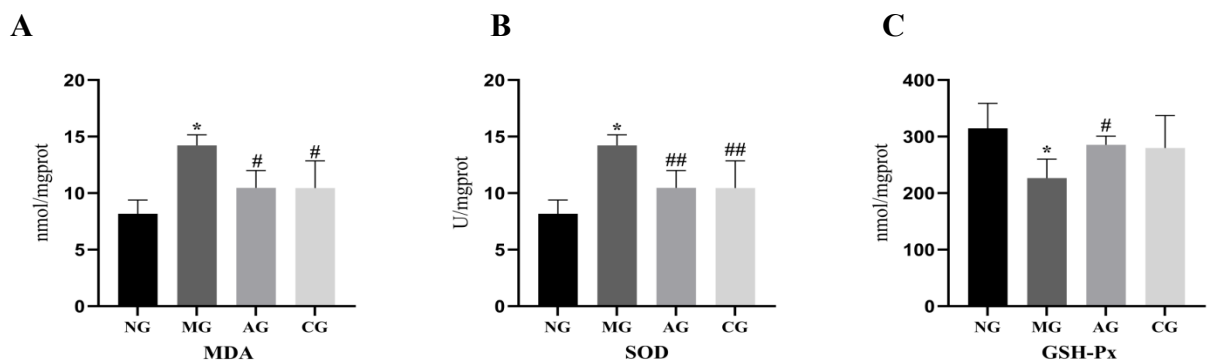


Figure 1. Comparison of oxidative stress markers in epididymal tissue. (A) Malondialdehyde. (B) Superoxide dismutase. (C) Glutathione peroxidase. Note: * $p < 0.01$ (versus NG); # $p < 0.05$ (versus NG); ## $p < 0.01$ (versus MG). Abbreviations: AG: Acupuncture group; CG: Curcumin group; MG: Model group; NG: Normal group.

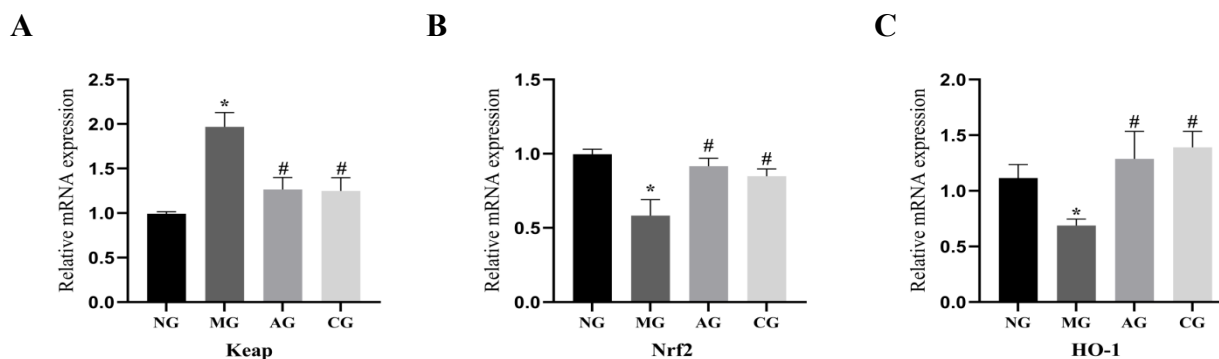


Figure 2. mRNA expression of (A) *Keap1*, (B) *Nrf2*, and (C) *HO-1*. Note: * $p < 0.01$ (versus NG); # $p < 0.01$ (versus MG). Abbreviations: AG: Acupuncture group; CG: Curcumin group; MG: Model group; NG: Normal group.

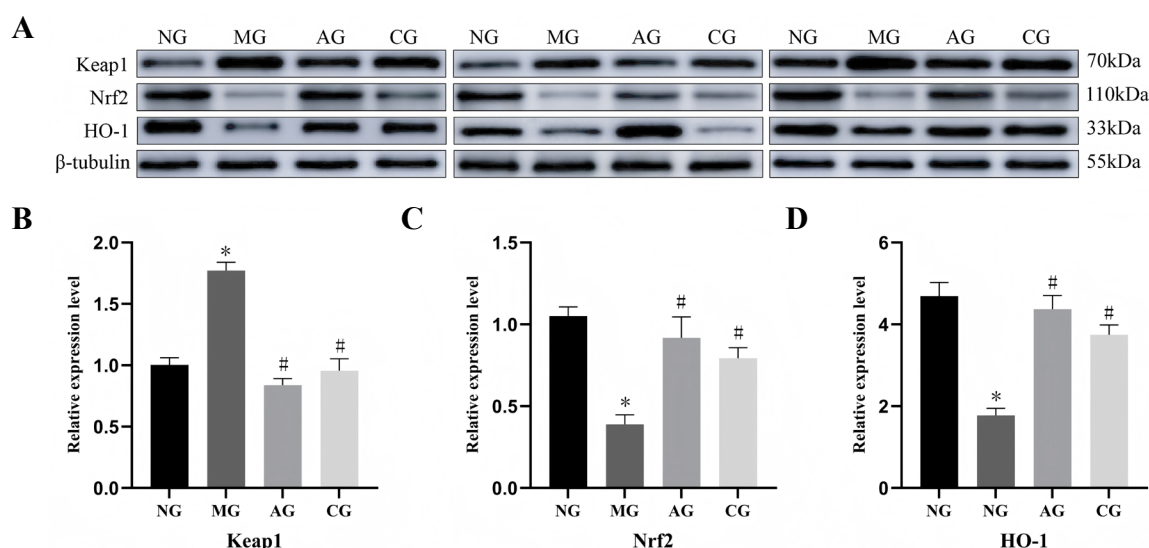


Figure 3. Protein expression of Keap1 (70 kDa), Nrf2 (110 kDa), and HO-1 (33 kDa). (A) Western blots of the proteins (three replicates). Relative expression of (B) Keap1, (C) Nrf2, and (D) HO-1.

Note: * $p < 0.01$ (versus NG); # $p < 0.01$ (versus MG).

Abbreviations: AG: Acupuncture group; CG: Curcumin group; HO-1: Heme oxygenase 1; Keap1: Kelch-like ECH-associated protein 1; MG: Model group; NG: Normal group; Nrf2: Nuclear factor erythroid 2-related factor 2.

unlike curcumin, which acts as a direct electrophilic inducer by chemically modifying Keap1 cysteine residues to liberate Nrf2, electroacupuncture may additionally engage indirect pathways. Low-frequency electrical stimulation at acupoints is known to trigger the release of endogenous neuropeptides such as substance P and calcitonin gene-related peptide. These mediators could, in theory, activate intracellular kinase cascades, including phosphoinositide 3-kinase/protein kinase B and protein kinase C, which have been reported in other systems to phosphorylate and activate Nrf2 independently of direct Keap1 alkylation. This remains a hypothesis and requires dedicated experiments using kinase-specific inhibitors and phospho-Nrf2 detection to be substantiated.

Mitochondrial integrity is widely acknowledged as a crucial factor in the development and progression of several human illnesses, including male infertility.³³ The significance of mitochondria lies in their role as the principal sites of ATP generation, supplying the metabolic energy required to support various cellular functions through coordinated catabolic and anabolic pathways.³⁴ The electron transport chain generates a proton gradient across the inner mitochondrial membrane, promoting ATP synthesis and generating the MMP. This leads to the formation of a proton gradient across the inner membrane, thereby producing the MMP. A reduction in MMP signifies mitochondrial impairment and malfunction, namely the failure to generate ATP.¹⁰ A substantial reduction in

MMP was noted in the MG. This suggests that ornidazole-induced oxidative stress in the epididymis compromised electron transport and led to functional deficiencies in rat sperm mitochondria, thereby decreasing progressive sperm motility. The acupuncture intervention significantly improved these changes, probably by reducing oxidative stress in the epididymis and restoring the disruptions in energy metabolism between mitochondria and the adjacent matrix. The maintenance of MMP demonstrated here has significant consequences for sperm survival signaling, extending beyond merely reflecting ATP production capacity. The disintegration of MMP is a pivotal event that triggers the mitochondrial permeability transition pore, leading to the release of pro-apoptotic factors, such as cytochrome c, into the cytoplasm. In the case of AZS, this cascade not only reduces motility but may also trigger caspase-dependent proteolysis of essential flagellar proteins. Considering that the Nrf2/HO-1 axis is recognized for its role in inhibiting mitochondrial permeability transition pore opening by reducing oxidative modifications of essential thiol groups in the pore complex, our findings indicate that acupuncture-induced restoration of MMP may simultaneously provide a protective effect against sperm apoptosis, thus maintaining the functional longevity of mature spermatozoa.

A fundamental inquiry in acupuncture research concerns how physical manipulation of the body's surface is perceived at the cellular level and converted into

meaningful biological signals. Acupuncture signaling is recognized as encompassing a complex, multi-tiered, multi-target network.³⁵ The insertion and manipulation of a stainless-steel needle generates a mechanical coupling action at the subcutaneous tissue. Research has shown that collagen fibers entwine with and firmly attach to the rotating needle body, generating traction forces that modify the local tension and structure of the extracellular matrix.³⁶ The physical alteration of the extracellular matrix is a significant initiator of mechanotransduction, transforming the mechanical energy from needle manipulation into a cascade of cellular responses.³⁷ Within the framework of this investigation, mechanical signals at acupoints are recognized as inducing local mast cell degranulation and promoting ATP release from keratinocytes and fibroblasts. These locally produced mediators act as intermediate messengers, transmitting signals through sensory afferent neurons to the central nervous system and, via somato-visceral reflexes, ultimately influencing the redox environment in distant target organs such as the epididymis. This preliminary biophysical observation establishes the crucial mechanistic link between the needle in the skin and the activation of the Nrf2 antioxidant pathway identified in sperm mitochondria.

Oxidative stress, defined by an imbalance between ROS production and antioxidant defense, is a critical etiological element in numerous disorders, including epididymal dysfunction and male infertility.³⁸ Emerging evidence suggests that acupuncture, a traditional therapeutic modality involving stimulation of specific acupoints, may exert beneficial effects, in part, through its regulation of redox homeostasis.^{39–41} Firstly, it reduces oxidant production by downregulating the expression and activity of ROS-generating enzymes.⁴² Secondly, acupuncture enhances endogenous antioxidant defense by upregulating key antioxidant enzymes, including SOD and GSH-Px⁴³, thereby elevating the overall antioxidant capacity of tissues. Thirdly, it attenuates oxidative damage markers, notably decreasing MDA and protein carbonyl levels, which serve as direct indicators of cellular injury.⁴⁴

The Keap1–Nrf2/HO-1 signaling pathway constitutes a well-established cellular defense mechanism against oxidative stress.^{45–47} Under oxidative stress, ROS selectively interact with reactive cysteine residues on the Keap1 protein, causing a conformational shift that inhibits its ability to bind and ubiquitinate Nrf2. As a result, stable Nrf2 accumulates and translocates to the nucleus, where it heterodimerizes with small Maf proteins and binds to antioxidant response elements in the promoters of target genes.⁴⁸ This transcriptional activation induces a significant increase in HO-1, hence augmenting cellular antioxidant

capacity and alleviating oxidative damage.^{48–50}

The knockout of the *Nrf2* gene in male mice results in age-dependent reproductive dysfunction, marked by heightened lipid peroxidation in the testes and epididymis, increased death of testicular germ cells, and a significant reduction in sperm motility.⁵¹ This study's Western blot and real-time PCR results indicate that acupuncture significantly decreased Keap1 expression while simultaneously increasing Nrf2 and its downstream effector, HO-1, at both the mRNA and protein levels in epididymal tissue. This synchronized regulation clearly indicates that acupuncture mitigates oxidative stress and enhances sperm mitochondrial function in ornidazole-induced AZS via activating the Keap1–Nrf2/HO-1 signaling pathway. The observed elevation of HO-1, a potent cytoprotective enzyme, together with the quantified rise in antioxidant enzymes and lipid peroxidation, elucidates the mechanism by which acupuncture augments cellular antioxidant capacity and reinstates redox balance.

Compared with curcumin, a well-known phytochemical activator of the Nrf2 pathway used as a positive control, acupuncture showed similar therapeutic effectiveness. Both therapies markedly restored the aberrant expression of Keap1, Nrf2, and HO-1, resulting in comparable improvements in sperm motility and MMP. This signifies a convergence on the ultimate shared mechanism of Nrf2-mediated antioxidant gene transcription. Nonetheless, a little difference in their methods may be present. Acupuncture has shown greater efficacy than curcumin in enhancing GSH-Px activity, a crucial enzyme in the glutathione system. This divergence indicates that although both treatments stimulate the Nrf2 axis, acupuncture may facilitate a more extensive or robust enhancement of the intrinsic enzymatic antioxidant network, possibly via supplementary regulatory mechanisms beyond the direct Keap1–Nrf2 interaction, including neuromodulation or influences on other stress-responsive pathways.

A crucial factor of the model utilized in this investigation is the pharmacokinetic profile of ornidazole. Ornidazole, a nitroimidazole derivative that may traverse the blood–brain barrier, is unlikely to confine its mitochondrial toxicity solely to the testes or epididymis^[52]. Systemic dosing may produce subclinical mitochondrial dysfunction in other metabolically active organs, such as the central nervous system and liver.^{53,54} This study was primarily designed to assess the localized therapeutic effects of acupuncture on sperm quality and epididymal redox equilibrium; nonetheless, we recognize the absence of data about mitochondrial function in off-target organs as a limitation. Considering that acupuncture is recognized for its neuroprotective properties and its ability to regulate

systemic oxidative stress, subsequent research should examine the potential activation of the Keap1–Nrf2/HO-1 pathway.

This study possesses several limitations. Initially, our data suggest a correlation between acupuncture and activation of the Keap1–Nrf2/HO-1 pathway; however, evidence of causation is lacking due to the absence of pathway-specific therapeutics, such as Nrf2 small interfering RNA or the selective inhibitor ML385. Secondly, while we observed improvements in MMP and reductions in oxidative stress following acupuncture therapy, direct evaluations of mitochondrial function, specifically ATP production and mitochondrial respiration, were not performed. The sham acupuncture group was omitted as well. Although curcumin served as a positive control to validate the involvement of the Keap1–Nrf2/HO-1 pathway, the absence of a sham control limits the ability to distinguish acupoint-specific effects from non-specific needle-related effects.

5. Conclusion

In summary, the present study elucidates that acupuncture treatment significantly improves sperm motility in a rat model of AZS. The fundamental mechanism driving this therapeutic action involves activation of the Keap1–Nrf2/HO-1 signaling pathway, which subsequently mitigates epididymal oxidative stress, restores sperm MMP, and amplifies cellular antioxidant defenses. These findings offer a definitive experimental and mechanistic foundation for using acupuncture in the treatment of male infertility linked to oxidative damage.

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Conflict of interest

The authors declare they have no competing interests.

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Writing—review & editing: Tian Xia, Qiang Geng

Ethics approval and consent to participate

The study protocol was approved by the Animal Ethics Committee of Yishengyuan Gene Technology (Tianjin) Co., Ltd. (Approval No.: YSY-DWLL-2023601).

Consent for publication

Not applicable.

Availability of data

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Appendix

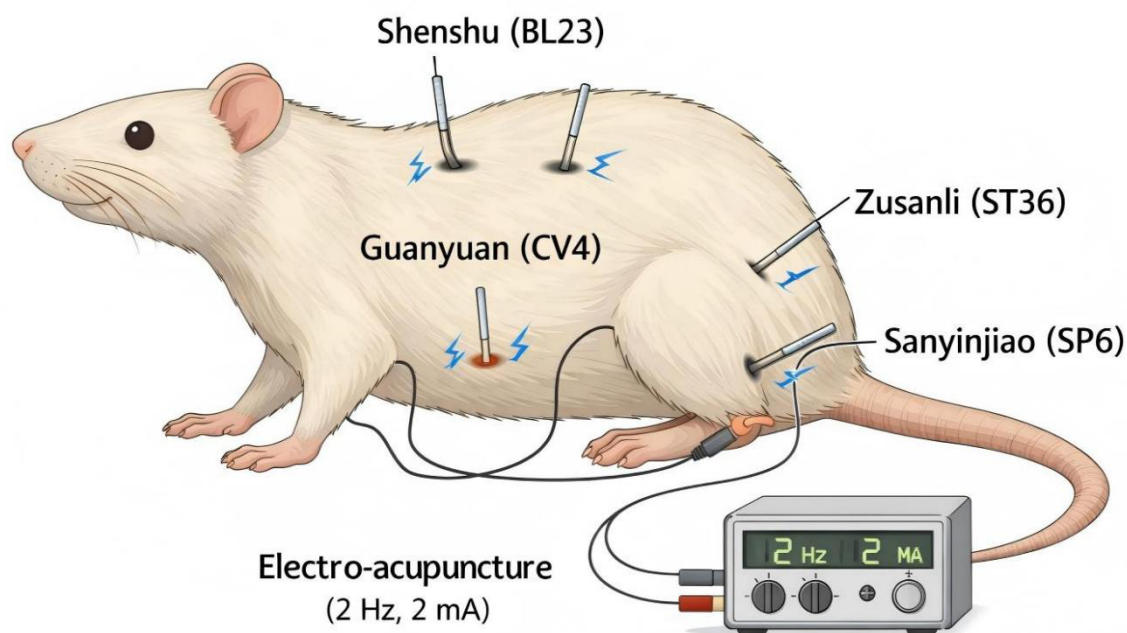


Figure A1. Schematic representation of acupoint locations and electro-acupuncture parameters used in the asthenozoospermic rat model

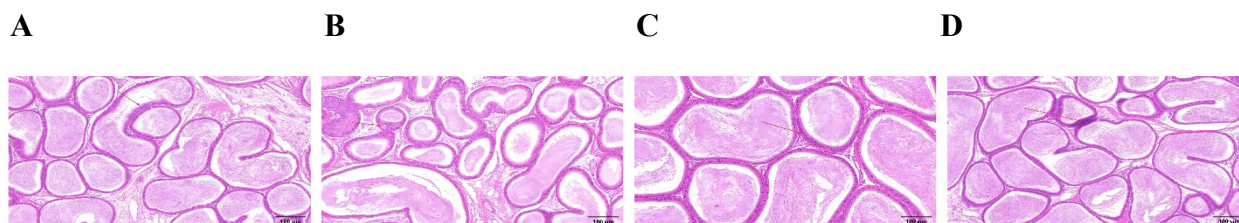


Figure A2. Hematoxylin and eosin staining of epididymal tissue. (A) NG; (B) MG; (C) AG; (D) CG. Scale bar: 100 µm; magnification: 100×. Abbreviations: AG: Acupuncture group; CG: Curcumin group; MG: Model group; NG: Normal group.