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Research paper

Moxa smoke: Is it a beneficial or hazardous factor for infertility? A preclinical study on sperm parameters and sex hormones in male rats☆,☆☆

Lei Wang[a], Li Han[b], Ping Liu[c], Jia Yang[a], Yingxue Cui[a], Hua Bai[d], Juntian Liu[a], Yuhai Huang[a], Jian Huang[a], Chang Huang[a], Baixiao Zhao[a,*]

[a] School of Acupuncture-Moxibustion and Tuina, Beijing University of Chinese Medicine, Beijing, People's Republic of China
[b] Institute of Health Preserving of Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing, People's Republic of China
[c] Beijing Electric Power Hospital, Beijing, People's Republic of China
[d] Xi'an Traditional Chinese Medicine Hospital, Xi'an, People's Republic of China

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ABSTRACT

Introduction: Moxibustion is commonly used as a traditional Chinese medicine treatment. The aim was to investigate how the exposure of moxa smoke influences sperm parameters, testis index and serum sex hormones in Wistar male rats.

Methods: 48 Wistar rats were randomly assigned, $n = 12$/group, to a control group or one of three moxa-smoke groups: M1 (low concentration, 0.4% of optical density), M2 (middle concentration, 2%) and M3 (high concentration, 15%). Rats in moxa smoke groups were exposed at the respective concentrations of M1 (0.4% of optical density), M2 (2%) and M3 (15%) for 12 weeks, at 5 days per week and 200 min per day. Dynamic analyses of sperm fluid were used to determine sperm concentration, progressive motility, sperm motility, linearity (LIN), wobble (WOB), straightness (STR), velocity of curved line (VCL), velocity of straight line (VSL) and velocity of average path (VAP). The testis index (TI) was measured, and serum sex hormones were determined by using ELISA.

Results: At the end of 12 weeks of treatment, when the control group was compared with the moxa-smoke groups, the moxa-smoke groups exhibited the following results: sperm concentrations were significantly higher in groups M1 and M3 ($p = 0.013$), progressive motility was significantly higher in M1 ($p = 0.006$), and sperm motility was significantly higher in groups M1, M2 and M3 (M1, $p = 0.003$; M2, $p = 0.044$; M3, $p = 0.008$). Compared to the control group, TI was also significantly higher in groups M1, M2 and M3 (M1, $p = 0.004$; M2, $p = 0.003$; M3, $p = 0.002$), as were serum total testosterone (TT), free testosterone (FT) and testosterone secreting index (TSI), respectively (TT, $p = 0.000$; FT, TSI, $p = 0.001$). Compared to M1, TT was significantly lower in groups M2 and M3 (M2, $p = 0.021$; M3, $P = 0.001$). The comparison of LIN, WOB, STR, VCL, VSL and VAP between groups showed no significant differences.

Conclusion: As compared to the non-moxa-smoke control group, low, middle and high concentrations of moxa smoke are effective to improve the male reproductive system in Wistar rats. More specifically, low moxa-smoke concentration seems slightly better than high concentrations for improving the sperm concentrations, progressive motility and sperm motility, TI, TT, FT, TSI of Wistar rats.

1. Introduction

The World Health Organization defines infertility as follows: Infertility is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse" [1]. Infertility may be caused by infection in the man or woman, but often there is no obvious underlying cause. In America, infertility affects approximately 10% of the population. The main cause of male infertility is

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* Corresponding author.
E-mail address: baixiao100@vip.sina.com (B. Zhao).

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low sperm quality. There are many factors that influence the sperm quality, including age, masturbation, heat, physical trauma, chemicals, endocrine disruptors, medication, hormones and diet. While a few chemicals with known effects on fertility have been excluded from human consumption, we cannot know if others remain undiscovered. Environmental mutagens, which are difficult to avoid, are associated with decreased semen quality.

Acupuncture has been clinically found to increase in vitro fertilization (IVF) considerable success rate [2,3]. Patients who suffer from infertility and receive acupuncture regularly are often prescribed moxibustion for treatment by acupuncturists. Moxibustion is a therapeutic procedure involving ignited material (mugwort, usually *Artemisia vulgaris*, also called moxa) to apply heat to certain points or areas of the body surface for curing disease through regulation of the function of meridians/channels and visceral organs [4].

There are various kinds of moxibustion treaments, including acupuncture-moxibustion therapy, for example, warm needling moxibustion-a treatment often used in acupuncture clinics or hospitals, performed by placing an ignited moxa stick on the needle handle after insertion. *Artemisia vulgaris* is a tall herbaceous perennial plant growing 1–2 m (rarely 2.5 m) tall, with a woody root. The leaves, which are used to make moxa, are 5–20 cm long, dark green, pinnate and sessile, with dense white tomentose hairs on the underside. The leaves are the original material for making moxa. Tracing back to the history of moxibustion, other materials were also used for moxibustion, including: peach branches, mulberry twig (Ramulus Mori), bamboo shavings (Caulis Bambusae in Taenia), shrub chastetree fruit (Fructus Viticis) and etc. However, moxa floss burns easily, evenly and slowly, providing a mild, consistent heat and a fragrant smell. it feels soft and binds together easily to roll moxa sticks. Moxa heat can pass through the skin and goes deep. Importantly, moxa ash does not easily break away when burning. For these reasons, moxa (or mugwort) is considered the best material for moxibustion.

Some acupuncturists find that using moxibustion in combination with acupuncture greatly enhances the healing of infertility [5]. Some clinics reported additional use of moxibustion during acupuncture in Swiss, German, and Austrian fertility centers and have published randomized controlled trials [6]. It is used widely in acupuncture clinics throughout China and other Asian countries as a form of treatment for various diseases. There, institutes for traditional Chinese medicine and acupuncture-moxibustion, conduct research on the clinical uses of moxibustion, such as breech presentation [7,8], ulcerative colitis [9,10], cancer [11], stroke rehabilitation [12], pain conditions [13], constipation [14] and hypertension [15].

However, due to the concern of the potential toxicity of the smoke, some clinics and hospitals no longer supply moxibustion. Concerns about moxa smoke are similar to those referring to tobacco smoke and air pollutants. Many studies show that exposure to tobacco smoke and air pollutants are associated with adverse effects in the immune, nervous, cardiovascular and respiratory systems. Such polluting air particulates adversely affect heart rate, blood pressure, vascular tone, blood coagulation, the progression of atherosclerosis [16] and sperm [17,18]. Cigarette smoking, for example, affects sperm plasma membrane integrity [19]. Smoking (cigarettes/day and duration) has detrimental effects on sperm motility, viability, DNA fragmentation, seminal zinc levels, and semen reactive oxygen species levels, even in fertile men, and it is directly correlated with cigarette quantity and smoking duration [20]. Researchers have found that the quality of early life in the womb, especially fetal growth and whether the mother smoked, has an important effect later on young men's testicular health [21].

Components of moxa smoke are different from tobacco smoke. Tobacco smoke is a toxic and carcinogenic mixture of more than 5000 chemicals [22]. Another research [23] provided a list of 98 hazardous smoke components, based on an extensive literature search for known smoke components and their human health inhalation risks. However, for components of moxa smoke, one study [24] did a qualitative analysis on components of moxa combustion products by solid-phase microextraction-gas chromatography-mass spectrography (SPME-GC–MS). The original moxa material in this research was from Qichun, in Hubei Province, which was considered the best moxa material production place. And the moxa was collected in 2009 and offered by Lishizhen office in Qichun, processed by Nanyang Wolong Hanyi moxa floss factory, with a proportion of 3:1, which indicated that 1 kg of moxa floss is processed from 3 kg of leaves of mugwort. The research showed that there were totally 61 separate peaks, and among them 26 components were identified, which consisted of three parts: components with furan structure at 0–10 min, aromatic compounds at 10–40 min, and esters, alkanes or hydroxyl-containing compounds at 40–70 min. The products of moxa combustion are mostly essence and aroma, and a few of them will have some toxicity in a higher concentration. Systematic toxicology research [25] has concluded that on moxa smoke have manifested that moxa smoke was not hazardous waste, and that the security of moxa smoke was up to the concentration. Low concentration (10% of optical density) had no significant effect, while middle (40%) and high concentration (70%) damaged the lungs and respiratory system, affected metabolism, immune system and showed no significant effect on the blood cells, liver and kidney function, and that the testis index among Groups L (10% of optical density), M (40%) and H (70%) was significant higher as compared with control group (without exposure of moxa smoke) 12 weeks after exposure period (25 weeks, 20 min per day, and 7days per week). Additionally, the testicles of some rats in the control group were atrophied and accompanied with low spermatogenic function. However, all the testicles in Group H were normal and the organic structure was complete. Analysis found that it is possible for moxa smoke to retain the function of testicles.

Modern research on the mechanism of the moxibustion focuses on the thermal effects, radiation effects, and pharmacological actions of moxa and its combustion products [26]. Other studies have explored the safety and effect of moxa smoke [27–31]. The mechanism of moxa smoke might be pharmacological and/or psychological [32]. Our hypothesis is that the mechanism of moxa smoke on reproductice system is similar to aromatherapy, the odor interacts with and affects the autonomic nervous system/central nervous system and/or endocrine systems. However, to our knowledge, the influence of moxa smoke on male reproductive system has not been sufficiently investigated. This paper was a part of toxicological research and evaluation of the safety of moxa smoke on sperm parameters in rats.

Sperm parameters include sperm concentration, progressive motility, sperm motility, linearity (LIN), wobble (WOB), straightness (STR), velocity of curved line (VCL), velocity of straight line (VSL) and velocity of average path (VAP). The factors of male reproductive function also encompass testis index (TI) and sex hormones, such as serum total testosterone (TT), serum free testosterone (FT) and testosterone secreting index (TSI). The purpose of this study was to determine how the exposure of moxa smoke influences sperm parameters, testis index and sex hormones (TT, FT, TSI) in Wistar rats.

2. Materials and methods

2.1. Experimental animals and study design

48 male Wistar rats, seven week of age, weight of 60 ± 10 g, were obtained from the animal center of Beijing Vital River

Laboratories (A Charles River Company). The animal license number is SCXK (Tianjin) 2012-0001. Rats were housed in individual cages with free access to food and water. A controlled environment at a temperature of 20–24 °C, humidity of 50–60%, and 12-h light–dark cycle was maintained throughout the study. All procedures for animal experiments were conducted in accordance with the World Health Organization's International Guiding Principles for Biomedical Research Involving Animals and were approved by the local ethics committee of Beijing University of Chinese Medicine.

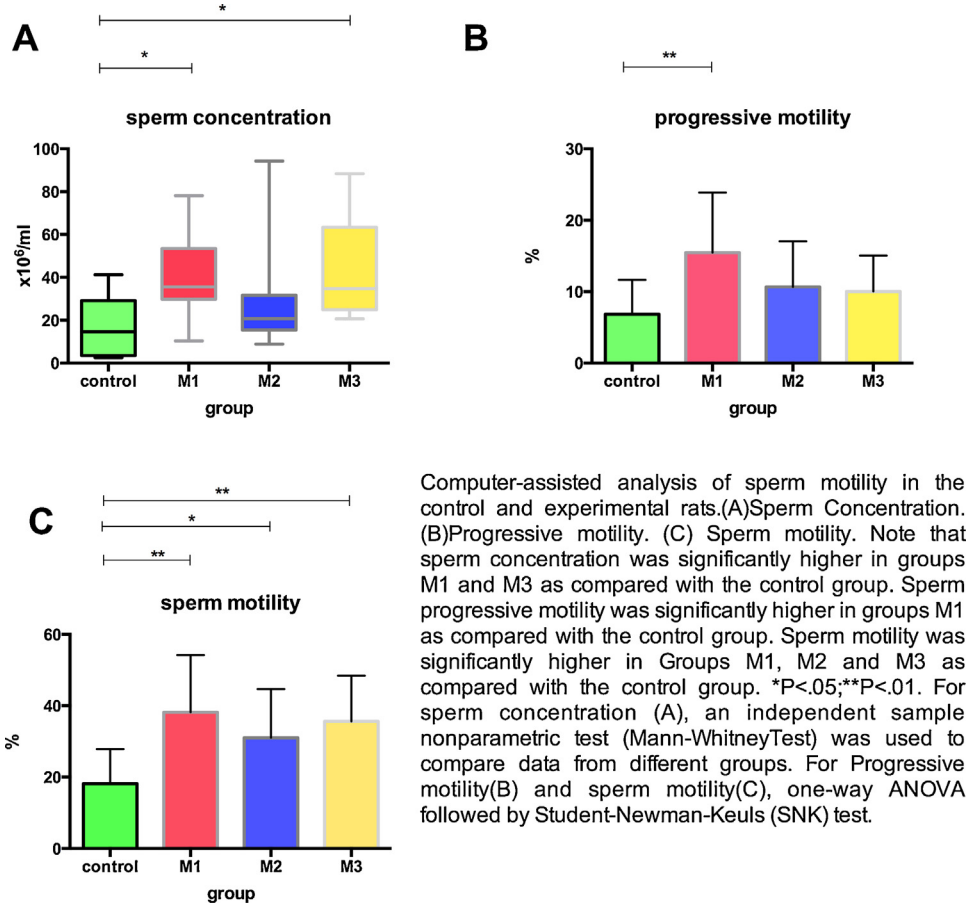
After acclimatization in the animal laboratory area for one week, 48 rats were randomly assigned ($n = 12$/group) to four groups: a control group (without smoke), M1 (low concentration, 0.4%), M2 (middle concentration, 2%), and M3 (high concentration, 15%) moxa smoke groups. The three moxa smoke groups were exposed to moxa smoke for 200 min, once a day, for five days a week over the course of twelve weeks; rats in the control group were not exposed to moxa smoke.

2.2. Main experimental materials and apparatus

Moxa smoke was generated by burning moxa sticks (three-year-old pure moxa, 1.8 cm × 20 cm × 10, Nanyang Hanyi Moxa Co., Ltd., China). The moxa sticks were produced with a proportion of 3:1, which indicates that 1kilogram of moxa floss processed from 3 kg of leaves of mugwort. The moxa smoke was controlled by use of a Dynamic Toxicant Exposure Cabinet (HOPE-MED 805 series) supplied from Tianjin HOPE Co., Ltd., China, which was used to monitor smoke concentration by optical density. The optical density refers to the proportion of moxa smoke particulate visible in a light beam. The cabinet was composed of an inner container, an outer container, a heating and cooling system, and an analogue and computer-based control system. Moxa was burned in a smoke generating device. Flow of moxa smoke was then controlled for exposure into the cabinet. The cabinet maintained desired stable concentrations of moxa smoke, as well as controlled test temperatures, humidity, pressure differences and oxygen concentrations, to guarantee the repeatability of the research.

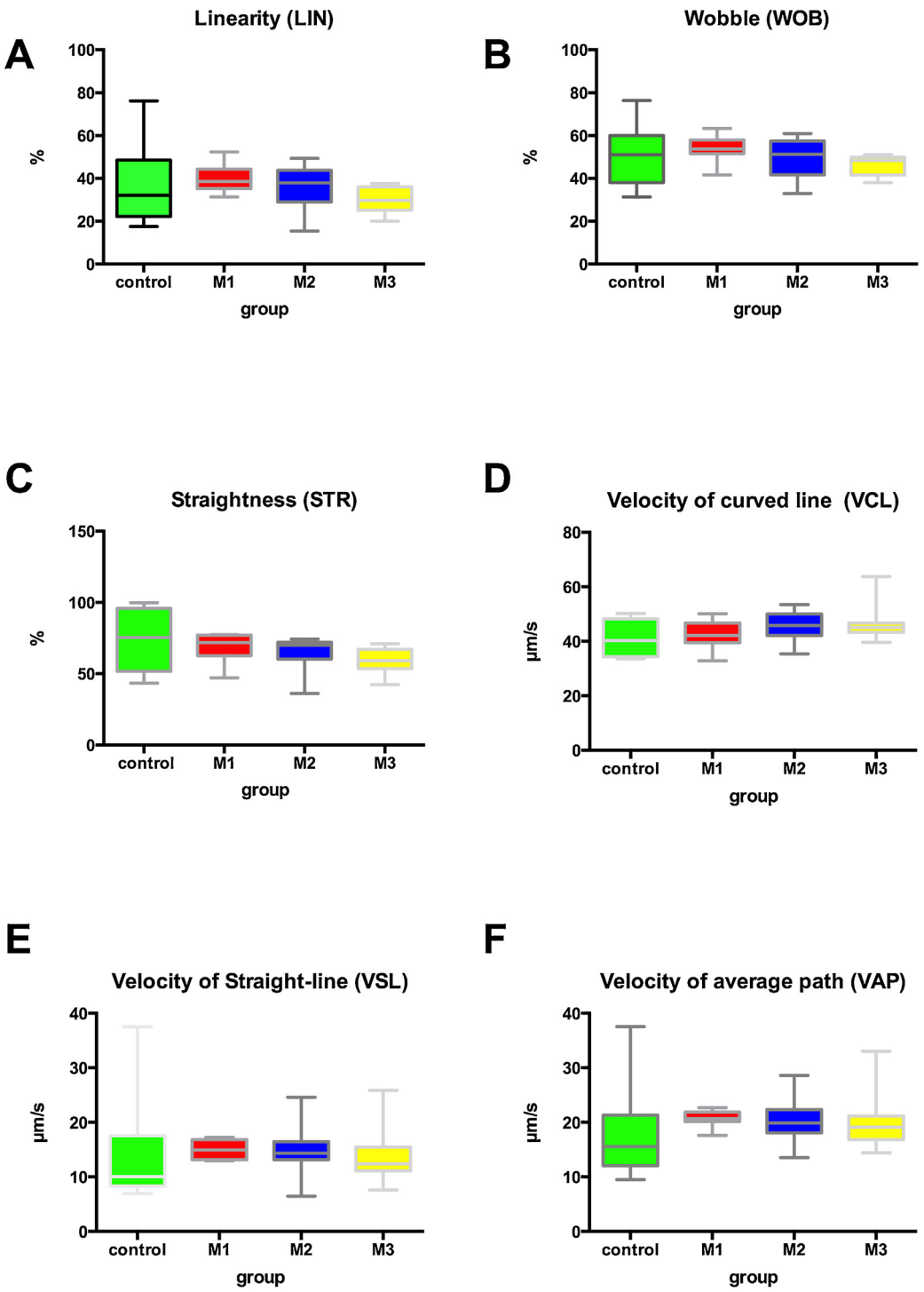
2.3. Experimental methods of different groups

The rats in the control group were set up first. Then, consecutively, the M1, M2 and M3 groups were treated. For the control group, all relevant parameters were set, such as concentration of oxygen, humidity, temperature and pressure. For M1, M2 and M3, first the moxa stick was burned, and then the same parameters were set as for the control group. Then, proceeding with the control group, 12 rats were put into the cabinet through the cabinet door, and left in the cabinet for 200 min with the door closed. For M1, M2 and M3, the same. Once when the concentrations of smoke were stable at 0.4%, 2% and 15% for each respective moxa group (including stability and equal consistency of all other parameter values), all parameters got the default value and stable, open the cabinet door, put in the 12 rats were put into the cabinet through the cabinet door, and left in the cabinet for 200 min with the door closed. After 200 min, all four groups were removed from their cabinets and returned to their cages.



Computer-assisted analysis of sperm motility in the control and experimental rats.(A)Sperm Concentration. (B)Progressive motility. (C) Sperm motility. Note that sperm concentration was significantly higher in groups M1 and M3 as compared with the control group. Sperm progressive motility was significantly higher in groups M1 as compared with the control group. Sperm motility was significantly higher in Groups M1, M2 and M3 as compared with the control group. *P<.05;**P<.01. For sperm concentration (A), an independent sample nonparametric test (Mann-WhitneyTest) was used to compare data from different groups. For Progressive motility(B) and sperm motility(C), one-way ANOVA followed by Student-Newman-Keuls (SNK) test.

Fig. 1. Computer-assisted analysis of sperm motility. (A) Sperm Concentration. (B) Progressive motility. (C) Sperm motility.



Computer-assisted analysis of sperm motility in the control and experimental rats. (A)Linearity(LIN). (B)Wobble (WOB). (C)Straightness(STR). (D)Curve-line velocity(VCL). (E)Straight-line velocity(VSL). (F)Average path velocity(VAP). Note that linearity, wobble, straightness, curve-line velocity, straight-line velocity and average path velocity were not significantly different in M1, M2 and M3 as compared with the control goup. An independent sample nonpatametric test (Mann-Whitney Test) was used to comare data from different groups.

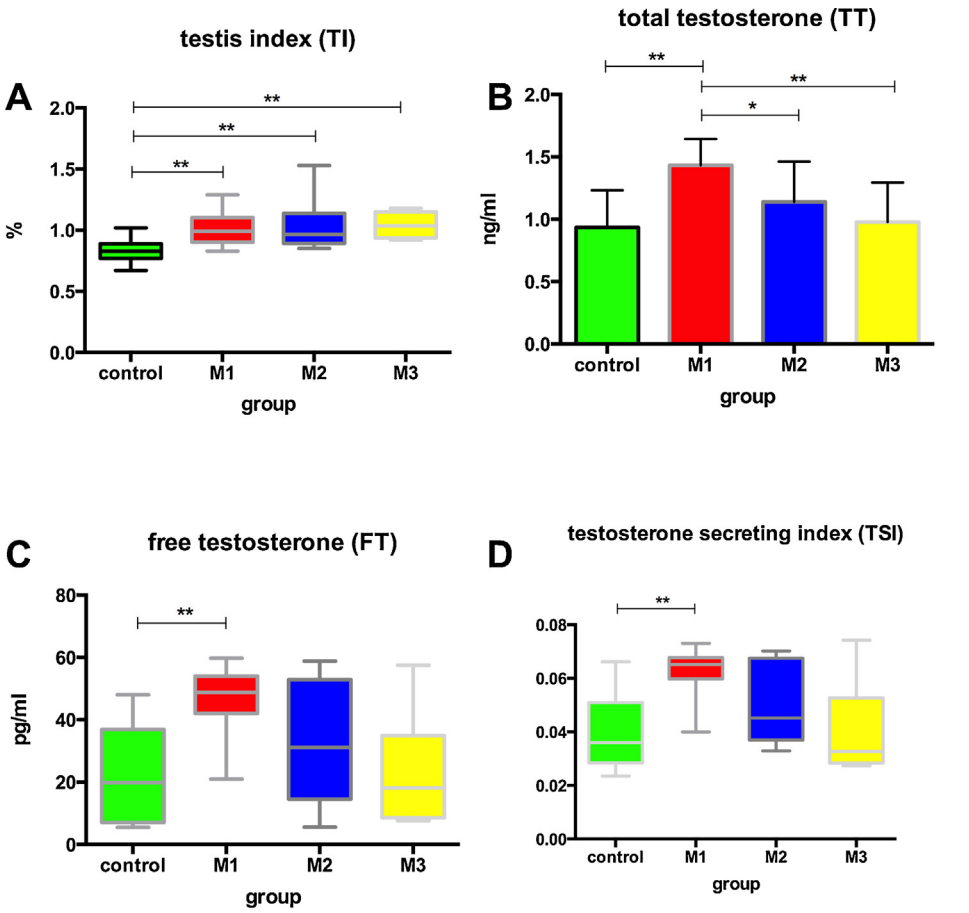
Fig. 2. Computer-assisted analysis of sperm motility. (A)LIN. (B)WOB. (C) STR. (D) VCL. (E) VSL. (F) VAP.

2.4. Sample collection and sperm parameters

Rats were weighed after the 12 weeks of treatment and then anaesthetized by 20% urethane. After collection of 5 ml blood via femoral artery, the rats were dissected. Epididymis was quickly removed and weighed. The two testis of each rat were quickly removed onto an ice board and weighed. The blood was collected into 10 ml of pro-coagulation tube, and was then centrifuged 1 h after collection at 22–25 °C. The settings for the centrifugal were 15 min at 4 °C with a rotation speed of 3500r per minute. The amber-colored liquid removed from the centrifuge was transferred into EP tube through, and was then put into. The serum in the EP tubes using a pipette, and then stored in liquid nitrogen, and refrigerated at −80 °C. Using enzyme-linked immune-sorbent assay kits of TT, FT, LH (produced by CUSABIO Bioengineering Limited Company, Wuhan, China), values of absorbance were strictly measured using a micro-plate reader at a wavelength of 450 nm. The testis index was computed with the weight of the testes divided by the body weight. The concentrations of TT, FT, LH were determined by comparing the O.D. of the samples to the standard curve.

In order to assess the sperm motility, one caudal epididymis was placed in Modified HEPESMedium (HM) (Lee and Storey, 1986) without $CaCl_2$ or BSA. The HMmedium contained NaCl (120 mM), KCl (2 mM), $MgSO_4 \cdot 7H_2O$ (1.2 mM), NaH_2PO_4 (0.36 mM), $NaHCO_3$ (25 mM), HEPES (10 mM), glucose (5.6 mM), sodium pyruvate (1.1 mM), penicillin (100 IU/ml), and streptomycin (100 μg/ml). The pH of the medium was adjusted to 7.1–7.3 by aeration with humidified air/CO_2 (19:1 v/v). Cauda was cut into 2–3 pieces and incubated at 37 °C for 10 min in CO_2 incubator. The sperm was gently filtered through nylon gauze and the filtrates (spermatozoa) were used to analyze sperm motility by computer-assisted sperm assay (CASA) with a sperm motility analyzer (IVOS version 10; Hamilton-Thorne Research, Beverly, MA, USA).

The following parameters of sperm motility were evaluated: sperm concentration, progressive motility, sperm motility, linearity (LIN, %), wobble (WOB, %), straightness (STR, %), progressive motility (%), curve-line velocity (VCL, μm/s), straight-line velocity (VSL, μm/s), average path velocity (VAP, μm/s). Sperm concentration, expressed as 10^6/ml, was determined by use of the hemocytometer method on two separate preparations of the semen sample.



Note: (A) Testis index(TI). (B)Total testosterone(TT). (C). Free testosterone(FT). (D). Testosterone secreting index (TSI). Compared to the control group, TI was significantly higher in groups M1, M2 and M3 (M1, p=0.004; M2, p=0.003; M3, p=0.002, **p<0.01), serum TT, FT and TSI were significantly higher (TT, ** p=0.000<0.01; FT, TSI, ** p=0.001<0.01) in M1. Compared to M1, TT was significantly lower in groups M2 and M3 (M2, *p=0.021<0.05; M3, **P=0.001<0.01). An independent sample nonpatametric test (Mann-Whitney Test) was used to compare data of TI,FT and TSI from different goups. For TT, one-way ANOVA followed by Student-Newman-Keuls (SNK) test.

Fig. 3. The results of (A)TI and sex hormones, including (B)TT, (C)FT and (D)TSI.

2.5. Statistical analysis

SPSS20.0 statistical software was used for data analysis. If data in all groups were distributed normally, the independent sample *t*-test was used to compare data from different groups, and all values were reported as mean ± standard error. If the data in all groups was not distributed normally, an independent sample nonparametric test (Mann–Whitney *U* Test) was used to compare data from different groups. And data were reported as median (25%value, 75%value). $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Sperm parameters

Computer-assisted sperm assay (CASA) revealed that sperm concentration was significantly higher in groups M1 [35.5700 (29.7500, 53.4600)] and M3 [34.7100 (24.9000, 63.3700)] as compared with control group [14.5850 (3.5550, 29.1525)] ($P = 0.013$) (Fig. 1A). Sperm progressive motility was significantly higher in groups M1 (15.46% ± 8.419%) as compared with control group (6.82% ± 4.806%) ($P = 0.006$) (Fig. 1B). Sperm motility was significantly higher in groups M1 (38.16% ± 15.996%) and M3 (31.64% ± 14.819%) as compared with the control group (18.19% ± 9.651%) (M1, $p = 0.003$; M3, $p = 0.008$). Sperm motility in Group M2 (31.01% ± 13.674%) was significantly higher as compared with the control group (18.19% ± 9.651%) ($p = 0.044$). However, there was no significant difference between sperm motility in groups M2 (31.01% ± 13.674%) and M3 (31.64% ± 14.819%) as compared with M1 (38.16% ± 15.996%) (M2, $p = 0.211$; M3, $p = 0.659$) (Fig. 1C).

For the sperm swimming patterns, computer-assisted sperm assay (CASA) revealed that linearity (LIN), wobble (WOB), straightness (STR), velocity of curved-line (VCL), velocity of straight line (VSL) and velocity of average path (VAP) were not significantly different in M1, M2 and M3 as compared with the control group (Fig. 2)

3.2. Testis index (TI)

TI was given with the weight of the two testes divided by the body weight. It was significantly higher in M1 [0.988% (0.902%, 1.107%)], M2 [0.968% (0.893%, 1.140%)], M3 [1.034% (0.937%, 1.149%)], compared with the control group [0.833%(0.767%, 0.885%)] (M1, $p = 0.004$; M2, $p = 0.003$; M3, $p = 0.002$). (Fig. 3A)

3.3. Sex hormones

3.3.1. Total testosterone (TT)

TT was higher in M1 (1.43236 ± 0.212565) as compared with control group (0.93467 ± 0.298195) ($p = 0.000 < 0.01$). However, total testosterone was lower in M2 (1.13883 ± 0.323917) and M3 (0.97592 ± 0.316524) as compared with M1 (1.43236 ± 0.212565) (M2, $p = 0.021$; M3, $P = 0.001$) (Fig. 3B)

3.3.2. Free testosterone (FT)

FT was significantly higher in M1 [50.9640(43.5868, 54.0100)] as compared with control group [19.8040(7.1325, 36.9278)] ($p = 0.001$). Compared to the control group, FT in groups M2 and M3 showed no significant difference. (Fig. 3C)

3.3.3. Testosterone secreting index (TSI)

TSI was given with the testosterone divided by the luteinizing hormone (LH). It was higher in M1 [0.0652(0.0598, 0.0677)] as compared with control group [0.0359(0.0285, 0.0509)] (TSI, $p = 0.001$). Compared to the control group, TSI in groups M2 and M3 showed no significant difference. (Fig. 3D)

4. Discussion

Air quality has become a global concern. The level of fine particulate matter, also called PM2.5, is reported in the air quality indexes as part of during the weather forecast. There were a lot of studies focusing on the influence of tobacco smoke. On the contrary, in our present study, we have demonstrated for the first time to our knowledge that moxa smoke can improve sperm concentration, progressive motility and sperm motility in rats. We also find that moxa smoke has no effect on LIN, WOB, STR, VCL, VSL and VAP. And also TI was significantly higher in groups M1, M2 and M3 than the control group. From the perspective of sex hormones, TT was significantly higher in Group M1 than the control group. However, with higher concentration than M1, TT was significantly lower in groups M2 and M3. It is interesting that the moxa concentration of Group M1 manifests the best result. Serum FT and TSI were both significantly higher than the control group.

In our study, moxa smoke improved the sperm concentration, progressive motility and sperm motility in rats. It is interesting to have this result discovered because it is important for male fertility and women pregnancy. It can be explained from perspective of traditional Chinese Medicine (TCM) that moxa smoke can tonify kidney yang and stimulate the function of yang of the whole body. In Chinese philosophy [4], yang refers to the masculine, active and positive principle (characterized by light, warmth, dryness, activity, etc.) of the two opposing cosmic forces into which creative energy divides and whose fusion in physical matter brings the phenomenal world into being. Kidney refers to a pair of organs located in the lumbar region, which store vital essence, promote growth, development, reproduction, and urinary function, and also have a direct effect on the condition of the bone and marrow, activities of the brain, hearing and inspiratory function of the respiratory system. Kidney yang deficiency pattern/syndrome refers to the pattern/syndrome arising when declined kidney yang fails to warm the body, marked by aversion to cold, cold limbs, listlessness, weakness and soreness of the loins and knees, premature ejaculation or impotence in men and frigidity or infertility in women, nocturia, whitish tongue coating and weak pulse at cubit (chi) section. According to the theory of TCM, yang energy (or heat) activates the function of different systems of the body and improves metabolism.

One study [33] about tobacco smoking concludes that tobacco smoke could seriously affect the quality of semen by increasing the content of reactive oxygen species in seminal plasma and sperm apoptosis. A related future study could investigate whether moxa smoke influences the content of reactive oxygen species in seminal plasma and sperm apoptosis.

We also find that moxa smoke has no effect on sperm swimming patterns, such as linearity, wobble, straightness, VCL, VSL and VAP. VSL, VCL and VAP are important parameters to evaluate sperm velocity, and an efficient index to reflect sperm motility ability [34]. The result of this research shows that moxa smoke has no bad effect on the aforementioned sperm swimming patterns. In short, moxa smoke has a positive effect on sperm motility, without any effect on VSL, VCL and VAP.

The weight of the two testes divided by the body weight gives the testis index. The testis index was significantly higher in M1 (0.4%), M2 (2%) and M3 (15%), compared with the control group. There was no significant difference about the body weight among the four groups. Therefore, it was the increase of the weight of the two testes, which caused the increase to the index. However, there was no significant difference between the testis indices of the three moxa smoke groups. It may be concluded that the concentration of

M1 at 200 min per day, 5 days per week for 12 weeks, provides the maximum effect (and that additional concentrations do not add to that effect). The moxa smoke exposure times in this study are similar to the amount of moxa smoke exposure for clinical acupuncturists or therapists. The results of this research correspond to previous research [25], which finds the testis index among Groups L (10%), M (40%) and H (70%) to be significantly higher as compared with a control group (without exposure of moxa smoke) 12 weeks after exposure period (25 weeks, 20 min per day, 7days per week). In our study, about the body weight, there was no significant difference between the body weights among the four groups. However, the previous research [25] concludes that the body weights in the 10th and 17th week in moxa smoke Groups M (40%) and H (70%) were significantly lower as compared with control group (without exposure of moxa smoke). There was no significant difference respective to the body weights during 6 weeks or 12 weeks after 25 weeks of moxa smoke exposure. In our study, the concentrations of moxa smoke were 0.4%, 2%, and 15%, which were much lower than 10%, 40% and 70% respectively. An explanation could be that the concentrations of moxa smoke in our study were not enough to influence the weight of the rats.

From the perspective of sex hormones, serum total testosterone (TT) was significantly higher in Group M1 than the control group. We believe this increase of TT to be the result of the increases of the testis indices. One research [35] about tobacco smoke concludes that heavy and long-term smoking repressed the synthesis of testosterone and decreased the level of L-carnitine in seminal plasma. Future studies on moxa smoke can explore how exposure of moxa smoke in different concentrations influences the synthesis of testosterone and the level of L-carnitine in seminal plasma. However, with higher concentration than M1, TT was significantly lower in groups M2 and M3 compared with M1. It is interesting that the best results are found from the moxa concentrations of Group M1-a concentration that (based on these findings) may be suggested for best treatment results.

It is important to measure the level of FT because this hormone is responsible for sexual traits. When the male reaches puberty, the body begins to produce more amount of free testosterone than before. When a man gets older, the testosterone levels can fall and cause health problems. In our study, serum FT is significantly higher in M1 as compared with control group. Men with low levels of free testosterone can lose their sex drive, suffer bone loss, or become infertile. Acupuncture is mostly combined with moxibustion for couples with fertility problems. There was a study [36] demonstrating that acupuncture and moxibustion increased pregnancy rates when used as an adjuvant treatment in women undergoing in vitro fertilization, when embryo implantation has failed. Consistent to the result of TT, comparing the result of the three moxa-smoke exposed groups, the testis index has no significant difference among M1, M2 and M3, which manifests that the concentration of M1 is the best concentration for male to improve male fertility.

The testosterone, which is secreted by the testicles, has a negative feedback on LH, which is secreted in the pituitary gland. TSI refers to the secretion ability of TT under a certain amount of LH [37]. In our study, TSI in Group M1 was both significantly higher than the control group. TSI is significantly higher in M1 as compared with control group, but no significant difference among Groups of M1, M2 and M3. The result of TSI corresponded to TT and FT, which manifests that the concentration of M1 is the best concentration for male to improve male fertility.

In our study, TT, FT and TSI were significantly higher as compared with control group (without moxa smoke). The odor might stimulate the olfactory nerves and the limbic system of the brain. The present study provides the role of moxa smoke on reproductive system.

We are aware of the limitations of the present study. Rats in Groups M1, M2 and M3 were exposed to moxa smoke for 12 weeks within the concentration of 0.4%, 2% and 15% respectively. It is still unknown that whether it will show the same result for the effect of moxa smoke on sperm parameters and sex hormones for a shorter period (6 weeks) or a longer period (24 weeks). An effort to replicate the results in another laboratory can provide confirmation to support the findings: whether male patients exposed to daily, low concentrations (0.4% of optical density) of moxa smoke could find enhanced activity and mobility in their sperm.

In summary, low (0.4% of optical density), middle (2% of optical density) and high concentrations (15% of optical density) of moxa smoke are effective to improve the male reproductive system in Wistar rats, but low concentration seems slightly better than high concentration in improving sperm concentration, progressive motility and sperm motility, testis index, TT, FT, TSI in Wistar rats.

Conflicts of interests

The authors declare that they have no knowledge of any conflict of interest.

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