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ORIGINAL RESEARCH

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By ameliorating redox imbalance, patients of oligoasthenospermia increased sperm acrosin activity after smoking cessation in Chinese

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Abstract

Background: The massive harmful effects of cigarette (tobacco) smoking on reproduction and fecundity are apparent. Even smoking cessation is often suggested for infertility patients by clinic doctors, while the impact of smoking cessation on semen quality in patients with oligoasthenospermia is uncovered. **Methods**: Ninety oligoasthenospermia patients with long tobacco smoking history were directed by andrology doctors to cease smoking, and their cessation was followed up for 3 to 6 months. The changes of semen quality after cessation in patients with at least 70% cessation ratio were evaluated by Paired-*t*-test for the changes in sperm concentration (SC), sperm progressive motility (PR), sperm volume (SV), sperm acrosin activity (SAA), total sperm count (TSC), normal sperm morphology rate (NSMR) and DNA fragmentation index (DFI). The cessation index was calculated for each patient, and its correlation with changes of semen parameters was analyzed. **Results**: Among 90 study cases, 81 were followed up successfully. Upon andrology doctors' routine instructions, only 19 (23.5%, 19/81) achieved a minimum requirement of 70% cessation ratio. In such a cessation level, only SAA has a significant difference after smoking cessation and a significant correlation was observed between the cessation index with the changes in SAA. Inceased glutathione (GSH) level and decreased reactive oxygen species (ROS) level were found in sperm cells and seminal plasma after smoking cessation. **Conclusions**: Andrology doctors' routine instructions could not accomplish an ideal level of smoking cessation. The intervene of tobacco smoking cessation for patients of oligoasthenospermia only resulted in a significant improvement of SAA by ameliorating redox imbalance. The goal of improving male reproductive function may be achieved by further improvement of smoking cessation strategies.

Keywords

Smoking cessation; Oligoasthenospermia; Sperm acrosin activity; Redox imbalance

Al mejorar el desequilibrio redox, la actividad de la enzima Acrosin espermatozoidal aumenta en pacientes chinos con oligoastenospermia después de dejar de fumar

Resumen

Antecedentes: Los enormes efectos nocivos del tabaquismo en la reproducción y la reproducción son evidentes. Incluso en pacientes infértiles, los clínicos a menudo recomiendan dejar de fumar, y el efecto de dejar de fumar en la calidad del semen de los pacientes con oligoastenospermia aún no se ha detectado. **Métodos**: El virólogo guió a 90 pacientes con oligoastenospermia con antecedentes de tabaquismo a largo plazo a dejar de fumar y los siguió de 3 a 6 meses. Los cambios en la calidad del semen después de dejar de fumar en pacientes con una tasa de dejar de fumar de al menos el 70% se evaluaron mediante una prueba *t* emparejada para detectar cambios en la concentración de espermatozoides (SC), el movimiento progresivo de espermatozoides (PR), el volumen de espermatozoides (SV), la actividad acrosina de espermatozoides (SAA), el número total de espermatozoides (TSC), la tasa morfológica normal de espermatozoides (NSMR) y el índice de fragmentación del ADN (DFI). Se calculó el índice de parada de cada paciente y se analizó su correlación con los cambios en los parámetros del semen. **Resultados**: De los 90 casos estudiados, 81 fueron seguidos con éxito. Solo 19 personas (23.5%, 19/81) alcanzaron el mínimo del 70% de la tasa de dejar de fumar, según las instrucciones habituales de los virólogos. Bajo este nivel de dejar de fumar, solo la SAA tiene diferencias significativas después de dejar de fumar, y hay una correlación significativa entre el índice de dejar de fumar y los cambios en la SAA. Después de dejar de fumar, los niveles de GSH en los espermatozoides y el plasma seminal aumentaron y los niveles de Especies de oxígeno activo (ROS) disminuyeron. **Conclusiones**: La orientación rutinaria de los médicos masculinos no puede alcanzar el nivel ideal de dejar de fumar. La intervención para dejar de fumar en pacientes con oligoastenospermia solo mejora significativamente la SAA mejorando el desequilibrio redox. Al mejorar aún más las estrategias para dejar de fumar, se puede lograr el objetivo de mejorar la función reproductiva masculina.

Palabras Clave

Dejar de fumar; Oligoastenospermia; Actividad de la enzima Acrosoma espermática; Desequilibrio redox

1. Introduction

The number of smokers in China is huge, reaching a quarter of the total number of smoking people in the world and the multitudes of smokers gradually becoming younger [1, 2]. It was reported by the National Health and Family Planning Commission that men aged 20–39 years accounted for up to 50% of all smokers in Chinese [3], meanwhile the reproductive period of men highly coincides with tobacco smoking habits. Smoking has been well known as an important risk factor for respiratory diseases and cardiovascular diseases [4–6], meanwhile smoking cessation can significantly reduce the risk of respiratory and cardiovascular diseases and slow the progression of chronic obstructive pulmonary disease (COPD) [4]. In a randomized clinical trial containing 1504 smokers, Gepner *et al*. [5] found that smoking cessation was able to increase the circulating high density lipoprotein concentration and may lead to a reduced risk of cardiovascular disease. Lei *et al*. [6] reported that smoking cessation had mortality reduction effects in approximately 50% of patients diagnosed with COPD with long-term smoking habit.

During last decades, the infertility incidence was on the rise, among those male factors accounting for 30% [7, 8]. Poor living habits including smoking [9–11], drug abuse [12, 13], alcohol intake [14–16], obesity [17, 18], malnutrition [19, 20] and exposure to toxic substances [21–25] can lead to a decrease in sperm quantity and quality. Reecha *et al*. [9] comprehensively analyzed 5865 samples from multiple studies and found that the sperm motility, total sperm count (TSC) and normal sperm morphology rate (NSMR) in smokers were significantly lower than those of no-smoking patients. In animal experiments, Hassan *et al*. [26] used cigarette smoke to artificially create model of male rats, and observed that the number of germ cells, Leydig cells and Sertoli cells was decreased significantly in male model rats.

It is noteworthy that previous studies on reproductive hazards of cigarettes mainly focused on the influence of smoking, while few studies about the effect of smoking cessation on male reproductive capacity. Santos *et al*. [27] observed that the sperm concentration and sperm motility of a 53-year-old male infertile patient improved after 3 months of smoking cessation, but the total number of sperm, NSMR and sperm DNA fragmentation index (DFI) did not change significantly. In clinical practice of our and other reproductive centers, andrology doctors usually treat oligoasthenospermia patients with drugs and instruct patients to quit smoking during pregnancy preparation. The Chinese Expert Consensus on the diagnosis and treatment of oligoasthenospermia (2021 edition) [28] issued by the Reproductive Medicine Professional Committee of the Chinese Medical Association proposed that “Although there is a lack of high quality evidence, a positive and healthy lifestyle such as healthy diet, physical exercise, cessation of smoking and alcohol can help improve sperm motility and improve the probability of pregnancy”. Indeed, the objective evaluation of the specific clinical effects of smoking cessation is still lacking.

In view of the above situation, we conducted an intervention study in patients with oligoasthenospermia who smoked tobacco for a long time. The withdrawal degree achieved by general guidance was observed, the influence of smoking cessation on objective indicators such as sperm quantity and

quality was analyzed.

2. Materials and methods

2.1 Study subjects

From June 2021 to December 2022, totally 90 patients attending andrology clinic and meeting the criteria were included in this study. The inclusion criteria were: (1) according to the fifth World Health Organization (WHO) Human Semen Examination and Treatment Laboratory Manual [29], semen examination was performed and sperm concentrations (SC) between 5–15 $\times$ 10^6/mL or progressive motility (PR) between 10–32%. (2) a long history of tobacco smoking, mean >10 cigarettes per day and time >3 years. (3) a clear intention to quit smoking. The exclusion criteria were: (1) reproductive system malignancies. (2) family history with hereditary diseases. (3) reproductive tract infection. (4) abnormalities in the testis/vas deferens/epididymis. (5) other substances of abuse, such as alcohol, caffeine, cannabis. After applying the exclusion criteria, 90 cases were included for the study.

2.2 Intervention measures

The international "5A" intervention [30] was used for smoking cessation of patients, including Ask, Advise, Assess, Assist and Arrange. "Ask" used a questionnaire, the number of patients' smoking was stratified by average 5, 10, 15, 20, 25 and 30 cigarettes per day. "Advise" and "Assess" warned the health hazards of smoking, especially on male reproductive health and the need to quit smoking during pregnancy. "Assist" followed up patients twice a week by phone or wechat within 3–6 months after starting smoking cessation, and recorded the performance. "Assess" as a quality control measure, the spouse was also asked about the situation of smoking cessation and while it was inconsistent with the patient's feedback of smoking cessation, the patients were excluded from the study.

2.3 Semen routine analyses and sperm morphological analyses

After 2–7 days abstinence, samples of semen were collected by masturbation. After liquefying at 37 °C, by a computer-assisted semen analysis platform (Sperm Class Analyzer, MICROPTIC, Barcelona, Spain) semen routine analyses and sperm morphological analyses were performed following the "WHO laboratory manual for the examination and processing of human semen" (5th) [29]. In the Andrology Laboratory, the examinations were accomplished by experienced technicians.

2.4 Sperm acrosin activity (SAA) and sperm DNA fragmentation index (DFI)

SAA was determined with a commercial kit purchased from the Bred Life Science (YZB-2275, Shenzhen, China). Sperm DFI was assessed with the sperm chromatin structure assay (SCSA). The SCSA kit was purchased from CellPro Biotech Co., Ltd. (4890-025-K, Ningbo, China). The examinations of SAA and DFI were analysed as described previously [31].

2.5 Quantitative polymerase chain reaction (PCR)

By the manufacturer's recommendations, total sperms RNA was extracted with the Trizol reagents (15596026CN, Tianwei, Beijing, China). The QuantiTect SYBR Green PCR Kit (204143, QIAGEN, Shanghai, China) was used to perform Quantitative PCR was performed on an iCycler iQ PCR equipment (Bio-Rad, Olympus, Tokyo, Japan). The Primer 5.0 Software (PREMIER Biosoft International, Palo Alto, CA, USA) was used to design the primers. The primer sequences were as follows: spam1 forward: AAC GTC ACA CTA GCA GCC AA; spam1 reverse: AGG GAA GAG GCC TGA AAC AC; acrosin forward: GCA GTG CCA GGA GTA TGG TT; acrosin reverse: TTT TTG CCG ACG AAG CAG TG; β-actin forward: CAT GTA CGT TGC TAT CCA GGC; β-actin reverse: CTC CTT AAT GTC ACG CAC GAT. Quantitative PCR was conducted with the following conditions: (1) primary denaturation for 15 min at 95 °C, (2) 35 cycles of denaturation for 10 seconds at 95 °C and annealing/extension for 30 seconds at 60 °C. For each sample, triplicate were performed for Quantitative PCR, and as an internal reference the levels of β-actin mRNA were used to standardize the results. The $2^{\Delta CT}$ method was used to calculate relative change folds.

2.6 Immunofluorescence staining of sperms

Coated with 1% (w/v) gelatin (Sigma-Aldrich), the bottom of wells were dropped with sperms. With 4% paraformaldehyde fixed at room temperature for 30 min and 0.5% (v/v) Triton X-100 permeabilized for 10 min, the sperm cells were incubated for blocking with 1% albumin from bovine serum (BSA) serum at room temperature for 1 h. At 4 ℃ the rabit anti-spam1 (1:200, Abcam, Eugene, OR, USA, cat. no. ab196596) antibody was used for primary staining overnight. Washing with phosphate buffer saline (PBS) twice, at room temperature the goat anti-rabbit IgG (1:1000, ThermoFisher Scientific, Waltham, MA, USA, cat. no. A11070) with Alexa Fluor 488-labeled was added for secondary staining for 1 h. With 4′,6-diamidino-2-phenylindole counterstaining for 1 min, the sperms were observed by fluorescence microscopy (Nikon, Ti 2, Tokyo, Japan).

2.7 Reactive oxygen species (ROS) measurement

Following the manufacturer's instructions, the levels of ROS in sperms were determined by the 2′-7′-dichlorodihydrofluorescein diacetate kit (DCFH-DA, S0033S, Beyotime, Shanghai, China). Briefly, washing 3 times by polarization beam splitter (PBS), sperms were resuspended with 1.0 mL PBS containing 10 μM DCFH-DA. After incubating at 37 ℃ for 20 min in the dark, the Varioskan LUX microplate reader (Thermo Scientific, Waltham, MA, USA) was used to determine the fluorescent signals. As a value equivalent, the ROS level was determined by the standard ROS reagent kit in 1 $\times$ 10^6 sperms.

The Oxiselect™ assay kit (Cell Biolabs, San Diego, CA, USA) was used to analyze the seminal plasma ROS levels. After centrifuging for 5 min under 10,000 $\times$ g, the supernatants

were obtained as seminal plasma samples from semen. With 50 μL samples adding to the wells, the seminal plasma incubated with Catalyst reagentat for 5 min at room temperature. By adding 100 μL dichlorodihydrofluorescin solution to all wells, covering with foil, and incubating for 45 min at room temperature in the dark, the fluorescence was measured with a microplate reader. All seminal plasma samples were analyzed in duplicate.

2.8 Enzyme linked immunosorbent assay (ELISA)

After washing twice with phosphate buffer saline (PBS) and centrifugation, sperms were resuspended with 1 mL PBS. After ultrasonication at 20 Hz for 5 min with 3 seconds pulse and 3 seconds interval repeated cycles, the supernatants were collected by centrifugation at 12,000 rpm for 10 min. The supernatants glutathione (GSH) level was measured with GSH assay kit (Shanghaijining, China, cat. no. N20809). The contents of sperm GSH were standardized in μg/L/10^6.

After semen centrifuging for 5 min under 10,000 $\times$ g, the supernatants seminal plasma samples were used to measured GSH level with GSH assay kit above. The contents of seminal plasma GSH were standardized in μg/L.

2.9 Statistical analysis

SPSS (version 18.0; SPSS, Inc., Chicago, IL, USA) was used to perform data analysis. The Shapiro-Wilk test were uesd to assess for data normality. Paired *t*-test analysis and Pearson correlation analysis were used to compare the patient's own control data. Cessation rate = average smoking cessation per day/average daily smoking. Cessation index = cessation rate $\times$ Cessation duration. $p < 0.05$ was established as statistical significance.

3. Results

3.1 Situation of smoking cessation

This study included 90 male smoking patients with oligoasthenospermia, and a total of 81 patients completed the follow-up, 9 patients lost to follow-up. Sixty-two patients who did not reach the lowest smoking cessation rate of 70%, 19 cases ultimately completed follow-up visits and met smoking cessation requirements (19/81, 23.5%). The data of previous smoking situation, cessation rate, cessation duration, and smoking cessation index was shown in Table 1. The average of smoking cessation rate was 79.2%, and the average of smoking cessation index was 3.14.

3.2 Changes of the semen parameters after smoking cessation

Seven semen parameters, including SV, SC, PR, TSC, NSMR, SAA and DFI, were detected before and after 3–6 months of smoking cessation. The Shapiro-Wilk test showed that both data of the seven indicators followed a normal distribution. Paired *t*-test was used for the changes of semen quality before and after smoking cessation and the results were shown in Table 2. SAA has a significant difference after smoking cessation, and the other indicators were no significant changes.

3.3 Correlation between smoking cessation index and semen parameters

The cessation index for each patient was calculated based on the cessation rate and cessation duration (Table 1). Regression analysis was performed between the cessation index and the changes of semen parameter for patients, and the results were shown in Table 3. Cessation index was significantly associated with the changes of SAA.

3.4 Changes of the acrosome enzymes spam1 and acrosin after smoking cessation

A variety of phosphatases, glycohydrolases, esterases, proteinases and aryl sulfatases are in acrosome. Spam1 (sperm adhesion molecule 1) and acrosin were the best-studied type of acrosome enzymes. Acrosin proteolytic activity is often analyzed to represent the activities of all acrosome enzymes. Quantitative PCR detected the spam1 (Fig. 1A) and acrosin (Fig. 1B) mRNA levels in sperms increased significantly after smoking cessation ($p < 0.05$), suggesting transcriptional upregulation of these acrosome enzymes. Immunohistochemistry was performed by taking advantage of the commercially available spam1 antibody. The signals of spam1-staining were located in the sperm head (Fig. 2A) and positive counting sperms showed a significantly higher expression of spam1 in sperms after smoking cessation (Fig. 2B) ($p < 0.05$). Acrosome enzymes acrosin and spam1 were upregulated after smoking cessation validated by quantitative PCR and immunofluorescent staining of sperms.

3.5 Inceased GSH level and decreased ROS level in sperms and seminal plasma after smoking cessation

Our previous studies found redox imbalance may influence the level of acrosin activity. Thus, sperms' as well as seminal plasma' GSH and ROS levels were measured by ELISA and fluorescent spectrometry. The GSH levels in sperms and seminal plasma were both increased significantly after smoking cessation (Fig. 3A,B) ($p < 0.05$). Meanwhile, the ROS levels in sperms and seminal plasma were both decreased significantly after smoking cessation (Fig. 3C,D) ($p < 0.05$), which suggested that redox imbalance was ameliorated by the intervene of smoking cessation for patients of oligoasthenospermia.

4. Discussion

Previous studies have observed the effects of smoking (tobacco) on the indicators of semen, indicating that smoking has a negative impact on sperm quantity and quality [9, 32–34]. In animal experiments [26, 35–38], the establishment of tobacco smoking model usually used dynamic oxygen feeding equipment to collect high concentration of smoke and input it into a confined space. The single inhalation amount of animals obviously exceeds the amount of human smoking, and this poisoning method is not in line with the situation

TABLE 1. Smoking cessation status of 19 patients.

Patient NO.	Smoking time (yr)	Average daily smoking (branch)	Average smoking cessation per day (branch)	Cessation rate (%)	Cessation duration (m)	Cessation index
1	5	15	13	0.87	4	3.48
2	7	20	18	0.90	4	3.60
3	3	15	12	0.80	3	2.40
4	6	15	13	0.87	3	2.41
5	5	10	7	0.70	3	2.10
6	9	15	12	0.80	5	4.00
7	3	10	8	0.80	3	2.40
8	8	10	8	0.80	3	2.40
9	10	20	15	0.75	4	3.00
10	6	20	14	0.70	5	3.50
11	7	10	7	0.70	4	2.80
12	6	15	11	0.73	3	2.19
13	8	15	12	0.80	6	4.80
14	5	20	17	0.85	5	4.25
16	5	20	16	0.80	4	3.20
17	6	15	13	0.87	5	4.35
18	4	10	7	0.70	3	2.10
19	9	10	7	0.70	5	3.50
Mean	6.22	14.72	11.67	0.79	4.00	3.14

TABLE 2. The influence of smoking cessation on semen parameters.

Semen parameters	Mean ± SD		*p*	95% CI of differences	
	Before cessation	After cessation		Low limit	Up limit
SV (mL)	2.93 ± 1.26	3.05 ± 0.93	0.706	−0.54	0.78
SC (10^6/mL)	52.23 ± 38.09	56.89 ± 41.43	0.331	−5.41	14.73
PR (%)	25.02 ± 12.04	25.81 ± 3.66	0.882	−10.44	12.01
TSC (10^6)	159.26 ± 126.43	173.02 ± 132.13	0.518	−31.68	59.21
NSMR (%)	8.41 ± 3.47	7.33 ± 2.99	0.151	−2.62	0.46
SAA (mIU/10^6)	41.25 ± 21.40	57.91 ± 24.43	0.023	−0.11	13.45
DFI (%)	17.64 ± 10.27	13.97 ± 8.21	0.149	−9.32	−0.20

SV: sperm volume; SC: sperm concentration; PR: sperm progressive motility; TSC: total sperm count; NSMR: normal sperm morphology rate; SAA: sperm acrosin activity; DFI: DNA fragmentation index; SD: standard deviation; CI: confidence interval.

TABLE 3. Correlation between smoking cessation index and the changes of semen parameters.

	SV Changes	SC Changes	PR Changes	TSC Changes	NSMR Changes	SAA Changes	DFI Changes
r	0.518	−0.363	0.274	0.122	0.035	0.114	−0.470
p	0.084	0.246	0.388	0.706	0.725	0.043	0.123

SV: sperm volume; SC: sperm concentration; PR: progressive motility; TSC: total sperm count; NSMR: normal sperm morphology rate; SAA: sperm acrosin activity; DFI: DNA fragmentation index.

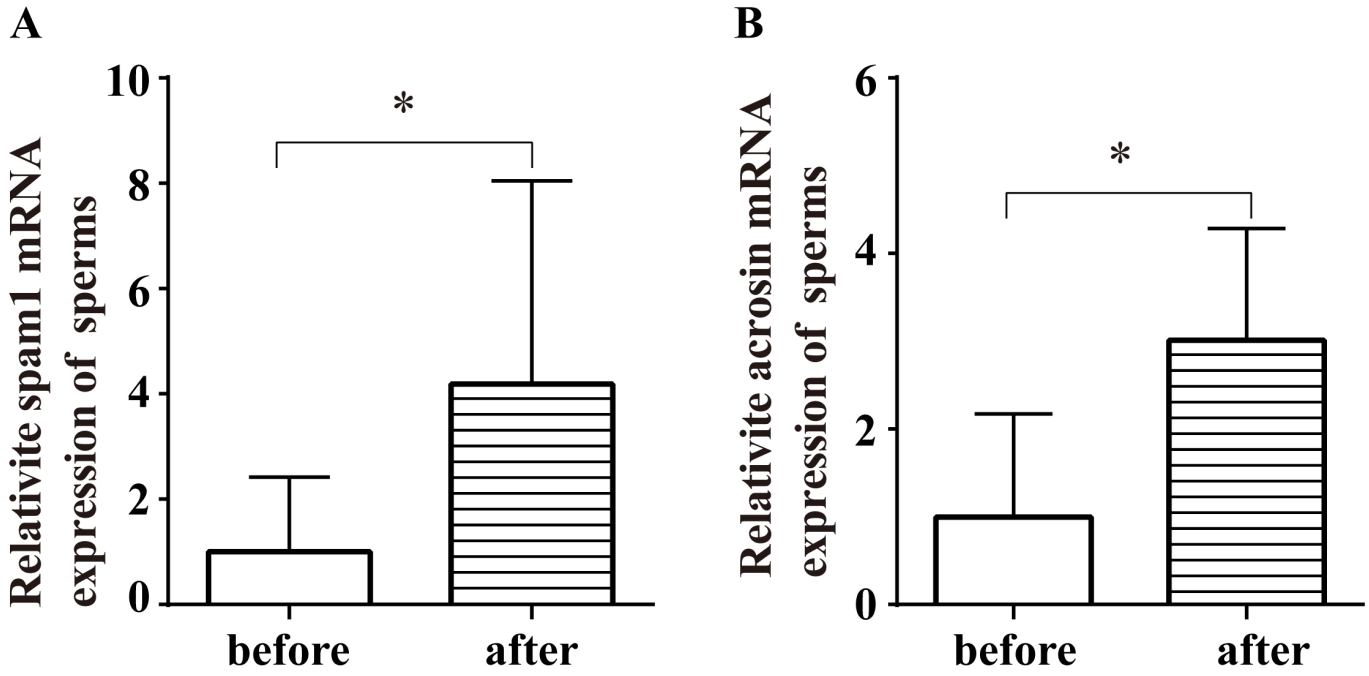

FIGURE 1. Increased mRNA levels of spam1 and acrosin in sperms after smoking cessation by quantitative PCR. (A) The mRNA level of spam1 was increased significantly in sperms after smoking cessation ($p < 0.05$). (B) The mRNA level of acrosin was increased significantly in sperms after smoking cessation ($p < 0.05$). *: $p < 0.05$; mRNA: Messenger RNA.

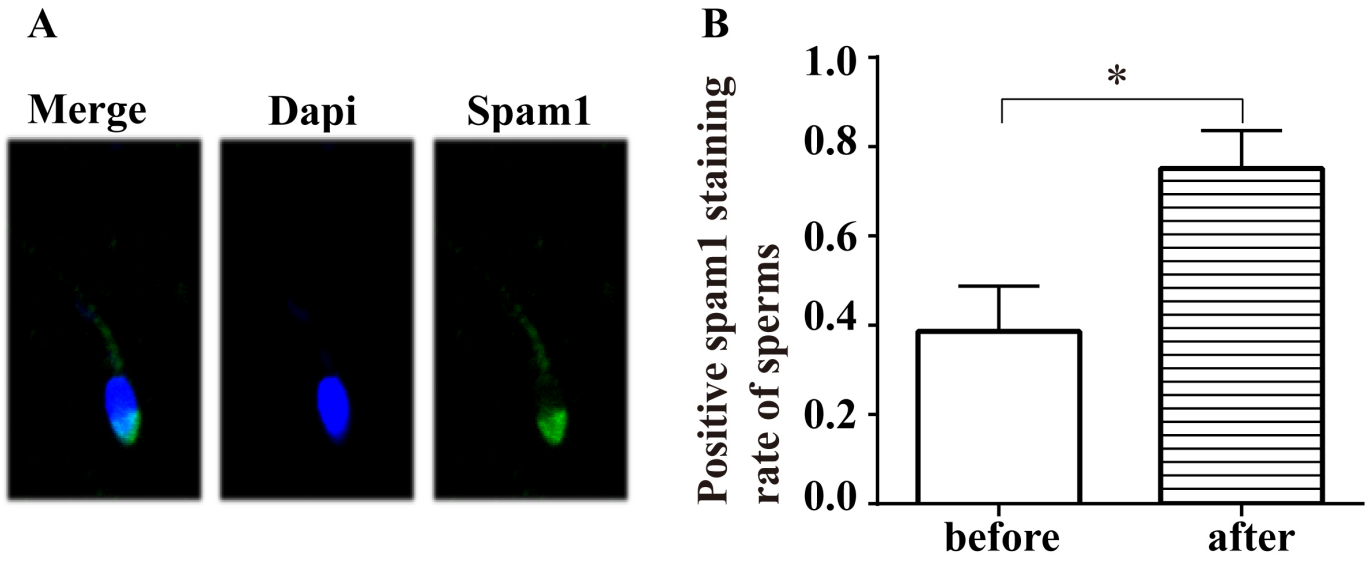

FIGURE 2. Increased protein level of spam1 in sperms after smoking cessation by immunofluorescence. (A) The immunostaining signals of Spam1 protein concentrated in the head of sperms. (B) After smoking cessation, the percentage of sperms with positive staining of spam1 increased significantly ($p < 0.05$). *: $p < 0.05$.

of human smoking. The smoking environment of human is relatively open, and the smoking rhythm is intermittent and automatic. Considering the difference in body weight between humans and rats, the relative exposure dose in humans was low. It is noteworthy that the negative effect of smoking cannot be simply explained as the reason that smoking cessation could improve reproductive function, especially for the short-term smoking cessation during pregnancy preparation, which is the focus of this study. We observed that under general supervision, male patients were difficult to achieve the ideal smoking cessation. During the 81 cases continuously tracked, 76.5% (62/81) of male patients failed to meet the minimum of 70% smoking cessation. Some scholars hold the view that the young smokers have more social smoking pressure than the older smokers, and the identity of partners as well as the health advantage of youth reduce the motivation to quit smoking [39–43]. Strategies and ways to improve smoking cessation guidance should be the direction of future efforts.

Currently, the examinations of semen are the main basis for evaluating male fertility ability and effects of treatment. The level of SAA changed significantly after smoking cessation, and there was a significant correlation between the cessation

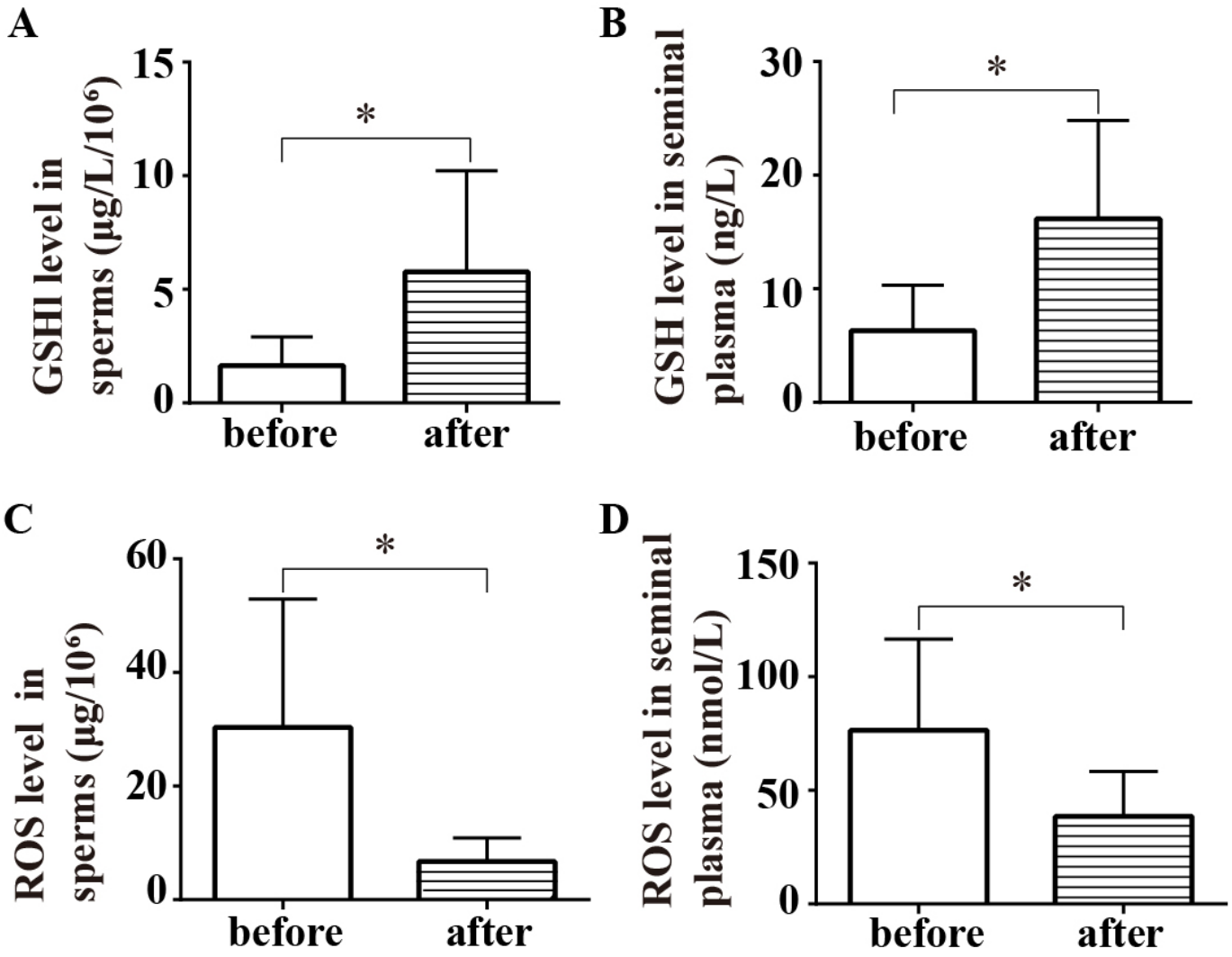

FIGURE 3. Inceased GSH level and decreased ROS level in sperms and seminal plasma after smoking cessation. (A) The GSH level in sperms increased after smoking cessation by ELISA ($p < 0.05$). (B) The GSH level in seminal plasma increased after smoking cessation by ELISA ($p < 0.05$). (C) The ROS level in sperms decreased after smoking cessation by fluorescence spectrometry ($p < 0.05$). (D) The ROS level in seminal plasma decreased after smoking cessation by colorimetry ($p < 0.05$). GSH: glutathione; ROS: reactive oxygen species. *: $p < 0.05$.

index and the changes of SAA levels by association analysis in this study. The negative results of other indicators showed that the degree of smoking cessation did not significantly improve the quantity and quality of sperm, suggesting that a more rigorous and longer period of smoking cessation was needed to achieve the improvement effect. Acrosin represents a series of trypsin-like serine proteinases in the acrosome of human spermatozoa [44, 45]. The activated acrosin could lyse the zona pellucida (ZP) of oocyte and facilitate the sperm to penetrate the ZP to accomplish a fertilization [46, 47]. Zhang *et al.* [48] observed a significant reduction in acrosin activity when the addition of carbonyl cyanide 3-chlorophenylhydrazone to semen samples induced elevation of reactive oxygen species (ROS) production and our previous studies found redox imbalance may influence the level of acrosin activity [31]. Taha *et al.* [49] reported that the ROS level of semen plasma was significantly higher in smokers than in non-smoking patients. We found increased GSH level and decreased ROS level in sperms and seminal plasma after smoking cessation. Therefore, smoking cessation may improve sperm acrosin activity by ameliorating redox imbalance. The significant improvement in acrosin activity after smoking cessation suggests that higher levels of smoking cessation may have a positive effect on conception outcomes.

There are several deficiencies in this study. First, the degree of smoking (tobacco) cessation is based on the feedback information of the patient and spouse, so it is difficult to confirm the facticity of patient's smoking cessation. The detection of carbon monoxide (CO) content in breath or the content of nicotine metabolites in blood can objectively reflect the smoking cessation situation [50, 51], but it is difficult to carry out due to the short retention time of CO as well as nicotine metabolites and the inconvenient operation of blood collection. Second, the number of samples in this study was small, and only 19 patients met the enrollment requirements, which may affect the accuracy of the results. Third, according to the current diagnostic criteria, the concentration less than 15×10^6/mL, or the PR less than 32% was regarded as oligoasthenospermia.

Considering that severe oligoasthenospermia may affect the sensitivity to the intervention, this study selected patients with mild oligoasthenospermia (sperm concentration between 5 to 15 $\times$ 10^6/mL or PR between 10–32%) as the study object, which could not represent all patients with oligoasthenospermia. In addition, the smoking cessation intervention in this study was limited to 3–6 months, and the improvement in reproductive function by smoking cessation may require a longer observation time.

5. Conclusions

This study is the first to evaluate the degree of general guidance by andrology doctor for patients with oligoasthenospermia, and analyze the effect of short-term and partial smoking cessation on multiple indicators of semen. Although the negative result was obtained, it explored a practical problem often encountered, which has certain reference value for andrology medical services. The results show that only further improvement of tobacco smoking cessation guidance strategies could achieve the goal of improving male reproductive function. This study is relevant for the medical service scenario, and is also suitable to explore the abstinence effects of poor lifestyle such as alcohol, coffee and electronic product dependence on male reproductive function.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

MYL, YKF, QWH and NHF—designed the research study; wrote the manuscript. MYL, YKF and YZ—performed the research. MYL and YKF—analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Ethics Committee of Women's Hospital of Jiangnan University approved this study (No: 2021-01-0120-01). All of the study subjects signed the informed consent form. All procedures performed involving human participants in experiments were carried out in accordance with the Declaration of Helsinki.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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