

EFFECTS OF JINKUI SHENQI AND WUZI YANZONG PILL ON  
SPERM MOTILITY AND SPERM DNA FRAGMENTATION RATE  
IN ASTHENOSPERMIALiangliang XU<sup>1</sup> , Chun Hong XIAO<sup>2</sup> , Hongyan CAO<sup>1</sup> , Jianfen YUAN<sup>1</sup> , Zhimei WU<sup>1</sup> <sup>1</sup> Department of Clinical Laboratory, Nantong Hospital of Traditional Chinese Medicine, Nantong, Jiangsu, China.<sup>2</sup> Department of Clinical Laboratory, Nantong Tumor Hospital, Nantong, Jiangsu, China.**Corresponding author:**

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**How to cite:** XU, L., et al. Effects of jinkui shenqi and wuzi yanzong pill on sperm motility and sperm DNA fragmentation rate in asthenospermia. *Bioscience Journal*. 2022, **38**, e38096. <https://doi.org/10.14393/BJ-v38n0a2022-54959>**Abstract**

This study was designed to compare the effects of Jinkui Shenqi and Wuzi Yanzong pill on sperm motility and sperm DNA fragmentation rate in patients with asthenospermia. 130 cases were randomly divided into an observation and control group (n=65). The control group was treated with the Wuzi Yanzong pill while the observation group with the Jinkui Shenqi pill. The sperm motility parameters rate (PR), semen concentration, sperm motility, DFI and  $\alpha$ -glucosidase, fructose, seminal plasma zinc (Zn), acid phosphatase (ACP) in seminal plasma biochemistry and other indexes of were observed. The biochemical indexes of seminal plasma of  $\alpha$ -glucosidase, fructose, Zn, ACP in two groups were significantly ( $p<0.05$ ) improved after treatment. Compared with the control group, the indexes of the observation group improved more obviously after treatment. Pearson correlation analysis of DFI and PR indexes in 130 patients before treatment showed that sperm DFI and PR percentage were negatively correlated in asthenospermia patients ( $r = -0.572$ ,  $P<0.05$ ). There was no significant difference in DFI, semen concentration, PR, and sperm motility between the two groups before treatment. The DFI, semen concentration, PR and sperm viability of the two groups showed a tendency to improve after treatment, and the effect of the observation group was less significant than that of the control group ( $p<0.05$ ). Two groups of patients have completed treatment successfully, no adverse events occurred during treatment. Jinkui Shenqi pill can effectively treat asthenospermia, which can effectively improve the effect of sperm motility in patients. It has less adverse reactions, safe and reliable, and is worthy of promotion.

**Keywords:** Asthenospermia. DNA fragmentation. Jinkui Shenqi pill. Sperm motility.**1. Introduction**

Because of the increase in modern environmental pollution, lifestyle changes, and other factors, the incidence of male infertility significantly increased. Male infertility is a very complex clinical syndrome. Sperm production, maturation disorders, sperm transport obstruction, and appendageal gonadal abnormalities caused by semen and sperm abnormalities are the most common causes of male infertility in clinical (Tsedon et al. 2007). China's male semen quality showed a downward trend, the incidence of infertility couples of childbearing ages has reached 10% -15%, of which male factors accounted for 55%. Treatment of male infertility is a long and lasting process. Due to many factors leading to male infertility and different treatment methods, it has not formed a set of effective and widely accepted diagnostic and therapeutic routines (Kobayashi et al. 2012). At the same time, because of the long treatment time, the patient's compliance with

the clinician also affects the treatment effect, so based on the traditional experience in diagnosis and treatment of infertility by traditional Chinese medicine, to explore new Chinese medicine therapy to treat male patients with asthenospermia is very important. Chinese medicine treatment of male infertility has good clinical efficacy, showing its advantages (Furuya et al. 2004). The regulation of traditional Chinese medicine is a kind of holistic regulation with definite curative effects and general non-toxic side effects. In recent years, great progress has been made in the experimental study of male infertility, which is of great significance to elucidate the mechanism of infertility and to explore the mechanism of traditional Chinese medicine treatment of infertility. However, the efficacy of different reports (Omu et al. 1998; Rolf et al. 1999; Hayashi et al. 2006) is not comparable, and it cannot fully reflect the defects of clinical practice. Traditional Chinese medicine holds that "kidney stores essence and main reproduction", which means that semen is the main substance in male reproduction. The vitality of the kidney essence has a direct impact on human growth and reproductive function, which is the main driving force for the promotion of reproduction and growth and development. Jinkui Shenqi pill is a classical prescription of traditional Chinese medicine essence from the "Golden Chamber", mainly used for the treatment of kidney-yang deficiency type disease. It was praised by the ancient physician, becoming the basic prescription for the use of Wenyang medicine commonly used for regulating male reproductive function and improving male fertility (Huang 2015). In the previous experiment, our research group found that the Jinkui Shenqi pill had a significant effect on the protection and promotion of induced differentiation of testicular spermatogenic stem cells. At the same time, it has a significant role in the promotion and protection of the Leydig cells which secret androgen mainly. In order to further explore the effect of Jinkui Shenqi pill on sperm motility and sperm DNA fragmentation rate in patients with asthenospermia.

## 2. Material and Methods

### General information

It was a Cross-sectional study design and the participants in a cross-sectional study were just selected based on the inclusion and exclusion criteria set for the study. 130 patients with asthenospermia were selected as research objects in our hospital from July 2019 to April 2020, aged 23-35 years, mean (31.5±1.2) years, course 1-4 months, mean course (2.7±0.8) months.

Inclusion criteria: Normal sexual life, no contraceptive measures, and two years without pregnant; dead sperm, oligospermia and asthenospermia lead to infertility; each ejaculation amount between 1mL to 6mL; immune infertility.

Exclusion criteria: Congenital infertility, including congenital azoospermia; infertility caused by genital diseases such as tuberculosis and other factors; genital malformations; women cause infertility; poor compliance; other reasons cannot receive the regular treatment; reproductive tract infection, varicocele patients; Research objects/patients who were not ready to stop alcohol use or who were acute addicted to alcohol were excluded.

Ethical requirements: all the subjects agreed and signed the informed consent, and the medical ethics committee assessed compliance with the requirements. 130 patients were randomly divided into an observation group and a control group, each group of 65 cases. There was no significant difference in gender, course, and other general data between the two groups ( $p > 0.05$ ).

### Treatment methods

The control group was treated with the Wuzi Yanzong pill (produced by Beijing Tongrentang Technology Development Co., Ltd.), 9g / time, 2 times / d, 4 weeks for a course of treatment, a total of 3 courses of treatment. The observation group was treated with Chinese medicine Jinkui Shenqi pill (produced by Beijing Tongrentang Science and Technology Development Co., Ltd.). Each time taking 8 pills and 4 weeks for a course of treatment, a total of 3 courses of treatment. Each pill is equivalent to the original drug 3g, the main ingredients: *Rehmannia*, *Yam*, *Cornus officinalis* (wine fried), *Poria cocos*, *Peony bark*, *Alisma orientalis*, *Ramulus Cinnamomi*, *Monkshood*, *Achyranthes bidentata* (without head), *Plantago asiatica* (salt

fried). Two groups were asked to stop smoking and drinking, live regularly, banned other drugs to improve the number and vitality of sperm.

### Detection index

Each subject was abstinent for 3-7d before and after treatment. The semen was collected by masturbation, placed in a sterile wide caliber cup, incubated in a 37°C water bath to observe and record the liquefaction time. The parameters of the semen routine were determined by using SUIJIA SOFTWARE computer-assisted sperm quality analysis system after liquefaction. Sperm DFI detection: sperm nuclear chromatin diffusion test was used to detect sperm DNA integrity. Spermatozoa chromosomes were stained with sperm DNA debris detection kit. 400 spermatozoa were counted under the phase-contrast microscope, and the number of abnormal spermatozoa with degradation, no halo ring, and small halo ring was recorded, then calculating sperm DFI. Seminal plasma biochemical determination: seminal fructose activity was determined by the chemical method with a reference value of 0.87-3.95 g/L. The  $\alpha$ -glucosidase was determined by the glucose oxidase method with a reference value of 35.1-87.7 U/mL. Semen plasma zinc was measured with a colorimetric method improved by Johnsen and Eliasson (1987) with a reference value of 0.8-2.5 mmol/L. Selenium acid phosphatase (ACP) detection was used as the conventional method of phenyl disodium phosphate with a reference value of 48.8- 208.6 U/mL. All reagents were purchased from Shenzhen Huakang Biomedical Engineering Co Ltd.

### Statistical analysis

All statistical analyses were performed using SPSS 21.0 software. The main statistical indicators were normatively tested in accordance with the approximate normal distribution. Categorical data and rate comparison were based on the student's t-test. The correlation was analyzed by Pearson correlation, using  $r$  to represent the correlation coefficient. A  $p$ -value of  $<0.05$  was considered statistically significant.

## 3. Results

### Comparison of biochemical indexes in seminal plasma before and after treatment

It was observed from the findings that the biochemical indexes of seminal plasma of  $\alpha$ -glucosidase, fructose, Zn, ACP in two groups were statistically significantly ( $P<0.05$ ) improved after treatment. Compared with the control group, the indexes of the observation group improved more obviously after treatment as shown in Table 1.

**Table 1.** Comparison of biochemical indexes in seminal plasma before and after treatment between two groups.

Groups	N	Fructose (g/L)		$\alpha$ -glucosidase (U/mL)		Zn (mmol/L)		ACP (U/mL)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control	65	1.05±0.21	1.58±0.33	40.32±5.29	56.28±4.55	0.65±0.13	1.14±0.34	66.06±4.79	103.37±12.18
Study	65	1.04±0.19	1.82±0.27	40.27±5.03	62.61±5.15	0.64±0.17	1.28±0.29	66.02±4.36	118.95±10.31
t		0.935	5.624	0.512	7.007	0.136	6.902	0.367	6.128
p		>0.05	< 0.05	> 0.05	< 0.05	>0.05	<0.05	>0.05	< 0.05

### Correlation analysis between PR and DFI

Pearson correlation analysis of DFI and PR indexes in 130 patients before treatment showed that sperm DFI and PR percentage were negatively correlated in asthenospermia patients ( $r = -0.572$ ,  $p < 0.05$ ).

## Comparison of the changes of DFI, semen concentration, PR, and sperm viability in two groups before and after treatment

There was no significant difference ( $p > 0.05$ ) in DFI, semen concentration, PR, and sperm motility between the two groups before treatment. The DFI, semen concentration, PR and sperm viability of the two groups showed a tendency to improve after treatment, and the effect of the observation group was less significant ( $p < 0.05$ ) than that of the control group, as depicted in Table 2.

**Table 2.** Comparison of the changes of DFI, semen concentration, PR, and sperm viability in two groups before and after treatment.

Groups	N	DFI (%)		Semen concentration ( $\times 10^6/\text{mL}$ )		PR (%)		Sperm viability (%)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control	65	20.25 $\pm$ 7.5	17.64 $\pm$ 6.3	35.12 $\pm$ 3.9	36.65 $\pm$ 5.0	18.97 $\pm$ 1.2	24.43 $\pm$ 1.0	27.58 $\pm$ 1.2	32.04 $\pm$ 3.2
Study	65	20.28 $\pm$ 7.4	15.32 $\pm$ 4.6	35.08 $\pm$ 3.9	39.08 $\pm$ 4.9	18.87 $\pm$ 1.1	31.28 $\pm$ 1.2	27.52 $\pm$ 1.1	42.19 $\pm$ 3.0
t		0.416	6.035	0.277	6.129	0.105	6.734	0.403	6.884
p		>0.05	<0.05	>0.05	<0.05	>0.05	<0.05	>0.05	<0.05

## 4. Discussion

Oligoasthenozoospermia is a very common type of male infertility, the incidence of which is on the rise, which is primarily divided into non-obstructive oligoasthenozoospermia and obstructive oligoasthenozoospermia (Zhang et al. 2014). In recent years, the incidence of male infertility has been observed to be high, but the cause of male infertility is complicated. Studies have shown that oligoasthenozoospermia is a major etiological factor of male infertility and characterized by spermatogenic disorder (Zhuo et al. 2019). Therefore, the treatment mainly takes nourishing yin for suppressing hyperactive yang and filling essence. The Monkshood in Jinkui Shenqi pill is okara hot, which is the first medicine of Wen Yang herbs. *Ramulus Cinnamomi* is spicy-sweet and gentle, which is the first choice of combination with Yang. Two drugs are used together, subsiding kidney yang deficiency, helping gasification of recovery, both are the king medicine (Qi et al. 2012). The kidney is hidden fire and dirty, which has originally Yin and Yang, and they can complement each other as a foundation, if only for Yang regardless of yin, then Yang cannot have attached to the place, so there is no way to play the role of promoting the warm. This is just like Zhang Jingyue said: "Good at adding Yang, will be in the Yin, while Yang gets the help of Yin and the biochemical infinite". So, this prescription is used Rehmannia to nourish kidney yin, accompanied by Dogwood, Yam to repair the liver and spleen, ensured the blood is abundant. They are both official medicines, combined imperial medicine and official medicine, tonifying the kidney to fill the essence, warming kidney to strengthen yang, strengthening yang through yin. Poria cocos, Alisma orientalis, and Plantago asiatica are used to remove water from the body, and Cassia twig is used to warm phlegm and water. Peony bark promotes blood circulation to remove stasis. Achyranthes bidentata can promote blood circulation and diuresis urination, with the combination of Cassia twig can relieve the blockage in the blood and relieve swelling. The five kinds of drugs supple through excretion. These drugs are combined, adding yang to remove moisture, nourishing yin to generator and water circulation, making Yang inspired, and returned to normal (Wang et al. 2013).

Relevant data show that male infertility is a multifactorial disease, in which spermatogenesis and maturation blocked are the main factors (Yang et al. 2009). About 85% of male infertility belongs to spermatogenesis disorder, which is characterized by abnormal semen quality and low spermatogenesis, decreased sperm quality and vitality, increased DNA fragmentation, and abnormal ascending morphology. The biochemical indexes of seminal plasma were changed correspondingly if the indexes of fructose,  $\alpha$ -glucosidase, and ACP changed. Studies have shown that seminal plasma fructose is associated with sperm

motility (Firestein et al. 2002). The results showed that there was a significant difference in fructose in patients with asthenospermia before and after treatment ( $p < 0.05$ ), suggesting that fructose as a sperm supply material has a direct association with sperm quality. Seminal plasma  $\alpha$ -glucosidase is a protease that hydrolyzes the egg zona pellucida. Thus, sperm obtain energy, pass through the cumulus and the zona pellucida, and fusion with the egg. It also promotes the release of kallikrein in the reproductive tract, thereby enhancing sperm motility and promoting sperm motility. The level of  $\alpha$ -glucosidase in patients with asthenospermia treated by the Jinkui Shenqi pill was significantly improved after treatment, and the sperm motility was significantly enhanced. The results showed that Jinkui Shenqi Pills had an obvious effect on the patients with weak semen, and the difference was statistically significant ( $p < 0.05$ ), indicating that the treatment of the Jinkui Shenqi pill was more obvious than that of Wuzi Yanzong pill. Prostate acid phosphatase (ACP) is a prostate secretory in the hydrolysis of phosphate glycoprotein, is a characteristic enzyme of the prostate, much higher than other tissues. Its function is to hydrolyze many phosphate monoesters in the acidic environment and transfer the phosphate to glucose and fructose to provide energy for sperm. Therefore, enzymes are related to sperm energy metabolism, and exercise. Studies have found that ACP has a significant effect on pregnancy in female rats, which is closely related to sperm quality (Yang et al. 2009; Amidu et al. 2012; Viveiros et al. 2014). This study showed that the difference was statistically significant in patients before and after treatment ( $p < 0.05$ ), and the difference was statistically significant in observation group compared with the control group ( $p < 0.05$ ), indicating that Jinkui Shenqi pill can improve the prostate function more than Wuzi Yanzong pill, and thus have a positive impact on the patient's sperm motility.

In recent years, the study of sperm DNA damage rate in patients with asthenospermia has become a hot spot in male infertility. The mechanism may be related to abnormal apoptosis, chromatin assembly abnormalities, or oxidative stress in the process of spermatogenesis. Some studies have shown that sperm motility, PR, and sperm DFI was significantly negatively correlated ( $p < 0.01$ ), which is consistent with the results of this study (Zhang et al. 2014). This study found that traditional Chinese medicine Jinkui Shenqi pill is more effective than Wuzi Yanzong pill, especially for sperm DNA fragmentation rate (DFI) treatment, showing that Jinkui Shenqi pills have a repair effect on the sperm DNA of patients with asthenospermia, and the improvement of DFI indicates that the integrity of the sperm due to the treatment of Jinkui Shenqi pill, the results similar to the previous conclusions. Reasons may be that sperm motility is related to the speed of sperm head movement, sperm movement is mainly affected by the tail swing mode, forming a curved or linear motion, and sperm mitochondria are the main organelle of sperm motility. When sperm DNA damaged, it can lead to abnormality in the number, structure, and function of sperm mitochondria. Therefore, DFI has been gradually applied to the diagnosis and treatment of asthenospermia, as a new index to evaluate sperm motility. It is an important supplementary parameter of sperm forward motion percentage in asthenospermia.

## 5. Conclusions

From the findings of this study, it can be summarized that, compared with Wuzi Yanzong pill treatment, Jinkui Shenqi pill can improve the essence of seminal plasma fructose,  $\alpha$ -glucosidase, Zn, ACP levels, regulate the prostate, epididymis, seminal vesicle and other functions, increase the patient's semen concentration, sperm viability, PR and reduce the DFI index, improve sperm motility. It has fewer adverse reactions, safe and reliable, which can be used as a reference for clinical treatment of asthenospermia.

**Authors' Contributions:** XU, L.: acquisition of data and drafting the article; XIAO, C.H.: acquisition of data and drafting the article; CAO, H.: analysis and interpretation of data and drafting the article; P; YUAN, J.: conception and design and critical review of important intellectual content; WU, Z.: conception and design and critical review of important intellectual content. All the authors have read and approved the final version of the manuscript.

**Conflicts of Interest:** The authors declare no conflicts of interest.

**Ethics Approval:** All the subjects agreed and signed the informed consent, and the medical ethics committee of Nantong Hospital of traditional Chinese Medicine assessed compliance with the requirements.

**Acknowledgments:** Not applicable.

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Received: 21 May 2020 | Accepted: 13 August 2021 | Published: 16 December 2022



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