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Peijingsu effectively improves sperm DNA integrity

Caixia Yuan[1,*], Haixia Song[1,*], Zhulin Wang[2] and Huaixiu Wang[3]

[1]Department of Reproductive Medicine, Shanxi Provincial Hospital, Taiyuan, China; [2]School of Environment and Engineering, Tianjin Industrial University, Tianjin, China and [3]Beijing Perfect Family Hospital, Beijing, China

Research Article

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Author for correspondence:
Huaixiu Wang. Beijing Perfect Family Hospital, Beijing, 100034, China.
E-mail: 976378008@qq.com

*These authors contributed equally to this work.

Summary

Intact human sperm DNA is an essential prerequisite for successful fertilization and embryo development. Abnormal sperm DNA fragmentation is a independent factor for male infertility. The objective of this study was to investigate the effects of Peijingsu, a health product, on the DNA integrity of human sperm. Peijingsu was administered for 15 days to 22 patients who had an abnormal sperm DNA fragmentation index (DFI). The DFIs before and after treatment were compared and analyzed using paired *t*-test. DFIs decreased significantly ($P = 0.0008$) after treatment, therefore it was concluded that Peijingsu effectively improved sperm DNA integrity in infertile patients who had an abnormal sperm DFI.

Introduction

Count, motility and morphology of sperm are traditionally tested as standard parameters for male fertility. With the advent of the DNA fragmentation assay, some infertile men were found to have a sperm DFI that was abnormally high, whereas standard sperm parameters were normal. Nearly 20% of patients with idiopathic infertility had high levels of sperm DNA fragmentation; chances of live birth and conception decrease drastically when sperm DFI is >30% (Hoeijmekers, 2001; Agarwal *et al.*, 2004). The zona pellucida of oocytes selectively binds sperm mature nuclei and normal DNA (Liu and Baker, 1992). When sperm DFI is >12%, the reproductive outcome after intrauterine insemination (IUI) is zero (Duran *et al.*, 2002). Although binding of sperm to the oocyte is bypassed by ICSI, per cent cleavage, embryo cell number and embryo grade are lower in patients who have severe DNA damage (Liu and Baker, 1992; Duran *et al.*, 2002; Morris *et al.*, 2002). Pregnant women whose male partners exhibit an abnormally high sperm DFI are prone to have idiopathic recurrent pregnancy loss when compared with controls (Iman *et al.*, 2011; Kamkar *et al.*, 2018). In addition, DNA damage in sperm is promutagenic. Small amounts of DNA damage in sperm could be repaired by pre- and post-replication repair mechanisms, but large-scale DNA damage cannot be repaired. This damage might result in major or minor congenital malformations, severe dysmorphogenesis or may lead to increased predisposition to certain cancers such as retinoblastoma (Cline and Hanawalt, 2003; Aitken *et al.*, 2007). It has been well recognized that the DFI has better diagnostic and prognostic capabilities than standard parameters and is a important independent factor for male infertility (Evenson *et al.*, 2002; Saleh *et al.*, 2003; Henkel *et al.*, 2004).

Now that the DFI is of such great significance, choosing sperm with a normal DFI for assisted reproductive technology (ART) might be beneficial. In IUI, however, *in vitro* fertilization and embryo transfer (IVF-ET) and intracytoplasmic sperm injection (ICSI), sperm selection are fundamentally based on sperm motility and morphology. To date, it has not been suitable to screen sperm with normal DFI and then use them for ART, therefore to enhance male fertility a practical direction would be to improve sperm DFI through medication. In this study, we investigated the effect of Peijingsu, a health product, on the DNA integrity of human sperm in infertile men.

Materials and methods

Materials and patients enrolled

Peijingsu (also called Sperm Nourishing Essence) was developed by UK Harvey Life Sciences Group Limited and is marketed in China by the Harvey Biotechnology (Beijing) Co. Ltd. It contains over 20 types of nutrition and trace elements for improving fertility of sperm. From 2 January 2019 to 1 May 2019, 25 infertile men aged 28–35 years old who had normal standard parameters of semen but a higher sperm DFI (above 30%) were enrolled in the present study. Their partners had apparently normal parameters such as patent Fallopian tubes, regular menstrual periods, and normal anti-müllerian hormone (AMH) and ovarian follicle counts. In total, 15 g of Peijingsu was administered to the patients twice daily for 15 days. Standard parameters of

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semen and sperm DFI were tested before and after treatment, respectively. This research was approved by the ethics committee of the hospital. Consent forms were signed by each male patient after detailed explanation about the research.

Semen collection and assay

Semen specimens were collected by masturbation after 48–72 h of sexual abstinence. After liquefaction, sperm concentration and motility were assessed using computer assisted semen analysis system (Weili Company Ltd, China). Smears of the raw semen were stained using the Diff-Quick Kit (Baxter Healthcare Corporation, Inc., McGaw Park, IL, USA) for assessment of sperm morphology following World Health Organization criteria (edition Vi). Normal values for sperm parameters were as follows: sperm concentration $\geq 15 \times 10^6$/ml; sperm with forward progressive motility ≥32% and sperm with normal morphology ≥4%.

DFI test

After standard semen analysis, the semen samples were frozen at −80°C. For the DFI test, the frozen semen were placed in a 37°C water bath until thawed. Then the samples were diluted with Tris–HCl/NaCl/EDTA (TNE) buffer to a concentration of sperm of 1×10^6/ml to 2×10^6/ml. Next, 200 µl aliquots of diluted samples were mixed with 400 µl of acid-detergent solution (0.08 M HCl, 0.15 M NaCl, 0.1% Triton X-100, pH 1.2) to induce DNA denaturation. Cell were stained 30 min later by adding 1.2 ml of 0.0005% (w/v) acridine orange (AO) solution (Polyscience, Warrington, PA, USA). Sperm cells were analyzed using flow cytometry (BD, Bioscience, USA) and an air-cooled argon laser operated at 488 nm with a power of 15 mW. In total, 5000 events were collected for each measurement. When excited with a 488 nm light source, AO intercalated with double-stranded DNA emits green fluorescence and AO associated with single-stranded DNA emits red fluorescence. The green fluorescence signal was collected through a 515–545 nm pass filter and red fluorescence through a 650 nm pass filter. The DFI was analyzed using FlowJo cytometry analysis software (Oregon, USA) and high DNA stainability (HDS) using SCSA Software (SCSA Diagnostics, Inc., Brookings Research and Technology Center, Brookings, SD, USA).

Statistics

The results before and after treatment were analyzed as paired data by *t*-test using GraphPad Prism 6.0 software (La Jolla, CA, USA). A P-value < 0.05 was considered as significantly different.

Results

From the 25 infertile men enrolled, three men did not return for the second test after treatment, therefore 22 male patients completed the investigation. As shown in Table 1, compared with the results before treatment, HDS was not significantly different but the DFI was significantly decreased ($P = 0.0008$). This result demonstrated that Peijingsu could improve sperm DNA integrity in infertile men wo had an abnormally high DFI.

Table 1. Comparison of sperm DNA integrity before and after treatment with Peijingsu

Items	Before treatment ($n = 22$)	After treatment ($n = 22$)	P-value
DNA fragmentation index (%)	49.69 ± 13.60	38.52 ± 13.90	0.0008
High DNA stability (%)	6.84 ± 4.1	6.35 ± 3.91	0.7564

Discussion

As a genetic template, the intactness of sperm DNA is vital for reproduction. During spermatogenesis and epididymal transit, about 85% of sperm nuclear histones are replaced by arginine-/cysteine-rich protamines for effective DNA condensation, making sperm transcriptionally and translationally inactive (Ward and Coffey, 1991; Shansi *et al.*, 2008; Selvam and Agarwal, 2018). During condensation, the thiol (–SH) groups of cysteine residues are progressively oxidized to form disulphide bonds, which provide protection to sperm DNA and are beneficial for DNA integrity (Bedford and Calvin, 1974) . DNA integrity of sperm is essential for normal fertilization, implantation, pregnancy and fetal development (Frazer, 2004; Benchaib *et al.*, 2007; Collins *et al.*, 2008). If DNA integrity is compromised (one manifestations is an abnormal DFI), DNA damage will occur and the fertility of sperm will decrease significantly.

In general, DNA damage is caused by incorrect DNA packaging, reactive oxygen species (ROS) attack and apoptosis.

During condensation of sperm DNA, double-stranded DNA undergoes torsional stress. Therefore, nicks and breaks in the DNA are created and repair takes place to maintain chromatin packaging (Sakkas *et al.*, 1995; Marcon and Boissonneault, 2004). Failure to repair these nicks and breaks leads to DNA damage including DNA fragmentation (Ward and Coffey, 1991; Sailer *et al.*, 1995; Aoki *et al.*, 2005).

Mitochondrial respiration is the main biological source of superoxide anion radicals under physiological conditions (Shansi *et al.*, 2008). Optimum amounts of free radicals in human semen enhance spermatozoa's ability to bind the zona pellucida, and undergo sperm capacitation, hyperactivation, acrosome reactions and oocyte fusion. Excessive levels of ROS lead to the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo), the major oxidative product of sperm DNA (Lopes *et al.*, 1998). This changes the tertiary structure and expression of proteins, and causes high frequencies of single-stranded and double-stranded DNA breaks, base modifications, deletions, frame shifts, cross-links and chromosomal rearrangements (Duru *et al.*, 2001). Both superoxide (O_2^-) and hydroxyl (OH^-) radicals are known to be mutagenic and cause chromosome deletions, dicentrics and sister chromatid exchange (Twigg *et al.*, 1998; Aitken and Krausz, 2001; Agarwal *et al.*, 2003; Sakkas and Alvarez, 2010). Conversely, spermatozoa with poor chromatin packaging and lower levels of disulphide cross-linking are susceptible to ROS attack (Steele *et al.*, 1999; Greco *et al.*, 2005). Abnormal chromatin packaging and pathological ROS form a vicious cycle, worsening DNA integrity (Padron *et al.*, 1997; Zini *et al.*, 2001; Saleh and Agarwal, 2002; Moustafa *et al.*, 2004; Peris *et al.*, 2007).

Both the intrinsic and extrinsic apoptosis pathways are activated in spermatozoa on continuous exposure to high levels of ROS and reactive nitrogen species. Activation of pro-apoptotic factors by ROS results in leakage of cytochrome *c* from the mitochondrial membrane, which in turn activates the intrinsic caspase cascade, resulting in sperm DNA damage (Sakkas *et al.*, 1999; Agarwal and Said, 2003; Shaha *et al.*, 2010; Gunes *et al.*, 2015).

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In addition to incorrect packaging, ROS attack and apoptosis discussed above, sperm must have some predisposed properties for DNA damage to occur. First, human spermatozoa have a high content of polyunsaturated fatty acid, which is easily oxidized into lipid peroxide (Evenson *et al.*, 2002; Henkel *et al.*, 2004). Lipid peroxide could in turn cause DNA damage and decrease the fluidity of the sperm membrane. Second, the ability to repair DNA in sperm is limited. In somatic cells, DNA lesions are repaired mainly with homologous chromosome as a template. In haploid sperm, there is no homologous chromosome. The alternative DNA repair mechanism, non-homologous end joining, is error prone and repair for DNA damage is not highly efficient (Hoeijmakers, 2001; Aitken and De Iuliis, 2007; Simon *et al.*, 2014). Due to the inherent factors mentioned above, sperm are susceptible to undergoing DNA damage.

The present study demonstrated that Peijingsu could improve sperm DNA integrity efficiently. The ingredients in Peijingsu include: lycopene, L-carnitine, linolenic acid, taurine, folic acid, lactalbumin, zinc, selenium and Chinese medicine herbs: ginseng, cordyceps sinensis, matrimony vine. Among these ingredients, lycopene is a strong antioxidant and could prevent the adverse effects of oxidants on spermatogenesis (Zini *et al.*, 2010; Ghyasvand *et al.*, 2015; Tvrda *et al.*, 2016). Taurine, L-carnitine, linolenic acid, zinc and selenium also have antioxidant capacities and hence could further decrease DNA damage caused by ROS (Abad *et al.*, 2013; Walczak-Jedrzejowska *et al.*, 2013). Lactalbumin could maintain antioxidant levels within body. *Cordyceps sinensis* is rich in superoxide dismutase (SOD), hypoxanthine and flavonoids. SOD is a important enzymatic antioxidant and could catalyze superoxide anions to H_2O_2 and O_2. H_2O_2 is in turn degraded into H_2O and O_2 by catalase. Hypoxanthine and flavonoids also have the capacity to combat ROS. Selenium is a key element in the active centre of glutathione peroxidase, which is an important enzymatic antioxidant. Ginseng contains some substances with adaptogen-like functions that could strengthen the reaction to mitigate multiple adverse stimulants. These combined functions could act towards antioxidant function and improve sperm DNA integrity.

Some studies have proposed that treatment with antioxidants has a positive effect on improvement of semen quality and DNA integrity (Lombardo *et al.*, 2011; Imamovic and Pinter, 2014). Vitamin C and vitamin E increase DNA decay even if ROS levels are reduced (Donnelly *et al.*, 1999). Agarwal and Allameneni (2004) reported that intake of oral vitamins (vitamin C and vitamin E) reduces DNA fragmentation, but also increases sperm decondensation by more than 25%. Menezo *et al.* (2007) also reported that medicating with vitamin C, vitamin E, β-carotene, zinc and selenium had similar adverse effects. An unwanted high degree of sperm decondensation can result in asynchronous chromosome condensation and may lead to cytoplasmic fragments in the embryo. No pregnancies have been observed following IVF or ICSI when sperm decondensation was >28%. The negative effect of vitamin C and vitamin E on chromatin packaging seems to originate from high reducing potential and is probably due to disulphide bond reduction in protamines. For example, vitamin C can reduce cystine to two cysteine moieties and therefore open interchain disulphide bridges. Antioxidants are therefore not recommended in men whose semen samples show a degree of decondensation over the threshold of 20%. It is interesting that, although the formulation of Peijingsu includes vitamin C and vitamin E, HDS, which reflects DNA decondensation, did not increase in the present study. The combination of vitamins with other ingredients such as lycopene, L-carnitine, linolenic acid, taurine, folic acid, lactalbumin, zinc, selenium and Chinese medicine herbs: ginseng, *Cordyceps sinensis* and matrimony vine could inhibit the side effects of vitamin C and vitamin E mentioned above.

This preliminary study demonstrated that treatment with Peijingsu could improve sperm DNA integrity, however the sample for the present study was small. The mechanisms that underly these Peijingsu effects need to be explored further based on a bigger sample size. As the ultimate aim of fertility treatment is to become pregnant and have a healthy child, pregnancy outcome should be investigated.

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Authors' contributions. Huaixiu Wang designed the experiment and wrote the manuscript. Caixia Yuan and Haixia Song performed the experiments. Zhulin Wang carried out the statistics calculation. All authors read and approved the final manuscript.

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Ethics approval. This study was performed in accordance with the guidelines for clinical experiments in Shanxi Provincial Hospital. The study protocol was approved by the ethics committee of Shanxi Provincial Hospital and a consent form was signed by each volunteer participating in the study.

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