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Prolonged Exposure to Polyvinylpyrrolidone Heightens DNA Breaks in Human Sperm

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Abstract

The preservation of sperm genetic integrity is pivotal for successful assisted reproduction. This study aimed to evaluate the effect of polyvinylpyrrolidone (PVP) on sperm DNA integrity by quantifying the mean number of DNA breakpoints (MDB). A total of 98 sperm samples were collected and subjected to comprehensive assessment of conventional sperm parameters, including concentration, progressive motility (PR), and sperm morphology rate. Furthermore, the MDB was assessed utilizing the TdT-SD Fluo-biosensor. The ultrastructure of the spermatozoa was meticulously examined using scanning electron microscopy and transmission electron microscopy. Exposure to PVP resulted in a substantial elevation in the mean number of DNA breakpoints within the spermatozoa, with discernible effects observed at the 10-minute mark and more pronounced effects evident at the 30-minute mark, when compared to the control group ($P < 0.001$). Electron microscopy analyses revealed perturbations in the plasma membrane and mitochondrial architecture of the spermatozoa. These findings suggest that prolonged exposure to polyvinylpyrrolidone (PVP), commonly used in assisted reproductive technology, may increase sperm DNA breakpoints, warranting careful consideration of exposure time in clinical practice.

Keywords Male infertility, Polyvinylpyrrolidone, TdT-SD Fluo-biosensor, Mean number of DNA breakpoints

Background

Infertility is a growing global health concern that affects the reproductive aspirations of millions of couples worldwide. Approximately 15% of couples worldwide are affected by infertility, with male factors contributing significantly to these cases[1]. Consequently, assisted reproductive technologies (ART) have

become a pivotal solution for infertility [2,3]. Intracytoplasmic sperm injection (ICSI) is a fundamental technique for treating male infertility. This procedure involves selecting morphologically normal, motile sperm under a microscope and injecting it into an oocyte [4]. To facilitate this procedure, polyvinylpyrrolidone (PVP) solution is commonly used in ICSI to reduce sperm motility[5,6], thereby immobilizing sperm for injection. Roychoudhury et al.[7] recommend PVP for sperm manipulation due to its potent antioxidant properties. Studies have shown that adding PVP to cryopreservation media significantly reduces sperm DNA fragmentation compared to conventional glycerol-based methods[8]. However, prolonged exposure to PVP may compromise sperm membrane integrity and damage chromosomal DNA[9]. Performing ICSI in patients with oligoasthenoteratozoospermia (OAT) is challenging because few morphologically normal sperm are available, potentially prolonging PVP exposure and increasing the risk of sperm damage [10]. Consequently, the effect of PVP on sperm structure and function—particularly DNA integrity—remains contentious. To better evaluate how PVP affects sperm DNA during ICSI, we introduced a novel probe technique.

This innovative method, which quantifies the mean number of DNA breaks per sperm (MDB), provides an effective and reliable measure of sperm DNA integrity [11-13]. This technique enables precise quantification of DNA breaks through analysis of over 12,000 sperm per sample. The quantitative data generated by this method facilitate evaluation of PVP-induced sperm DNA damage and provide deeper insights into its potential impact on DNA integrity. These findings offer valuable references for optimizing ICSI protocols, maintaining sperm DNA integrity, and supporting embryonic genetic stability.

Materials and methods

All methods were carried out in accordance with relevant guidelines and regulations.

Study design

This study utilized 98 semen samples collected from adult male patients (aged 20–40 years) at the Reproductive Medicine Centers of the General Hospital of Ningxia Medical University and the Affiliated Hospital of Inner Mongolia Minzu University. The study protocol was approved by the Ethics Committee of the General Hospital of Ningxia Medical University (Approval No.: KYLL2022-0144), and written informed consent was obtained from all participants.

Patient selection

The study cohort consisted of 98 semen samples, including 56 from patients diagnosed with asthenozoospermia (progressive motility < 32%, based on WHO 6th edition criteria) and 42 from normozoospermic controls (progressive motility ≥ 32% and normal morphology ≥ 4%, meeting all WHO reference standards). Exclusion criteria included a history of smoking, excessive alcohol consumption, drug use, prior varicocele surgery, other infertility-related conditions (such as cryptorchidism), exposure to substances or medications known to impair male fertility, urogenital infections, cancer, or endocrine disorders.

Sample preparation

After a sexual abstinence period of 2–7 days, semen samples were obtained by masturbation into sterile containers and allowed to liquefy at 37 °C for 30 minutes. Sperm concentration and motility were analyzed using computer-assisted semen analysis (CASA). Sperm morphology was assessed with a Diff-Quik staining kit (G1541; Beijing Solarbio Science & Technology Co., Ltd.) according to World Health Organization guidelines. Sperm viability was evaluated using

eosin-aniline black staining per the manufacturer's protocol: sperm stained dark red were considered non-viable (dead), while unstained sperm were classified as viable (alive).

Swim up

Transfer 1 ml of G-IVF medium into a test tube and add 1 ml of liquefied semen to the bottom. Tilt the test tube to a 45°and incubate for 30 minutes. After incubation, aspirate the supernatant (top layer) into a 15 mL centrifuge tube and centrifuge at 300 g for 5 minutes. Carefully discard the supernatant without disturbing the pellet. Wash the pellet with G-IVF medium and centrifuge again at 300 g for 5 minutes. Finally, suspend the pellet in G-IVF medium after centrifugation.

Effects of PVP on sperm

Take 50 µL of sperm suspension and mix it with 50 µL of 20% polyvinylpyrrolidone (PVP). Incubate at 37°C in a culture chamber for 0, 5, 10, 20, and 30 minutes, respectively.

Sperm Oxidative Stress Using Flow Cytometry

Sperm superoxide anion levels, as an indicator of oxidative stress, were measured by flow cytometry using a commercial detection kit (Cat. No. BKR 803; Wuhan Biocarry Technology Co., Ltd.). Intracellular reactive oxygen species (ROS) levels were assessed using the cell-permeable fluorogenic probe 2',7'-dichlorofluorescein diacetate (DCFH-DA). Non-fluorescent DCFH-DA is hydrolyzed within the cell and then oxidized by ROS—such as hydrogen peroxide, organic peroxides, and peroxynitrite—to produce highly fluorescent dichlorofluorescein (DCF). The assay was conducted in strict accordance with the manufacturer's instructions, with DCFH-DA applied at 10 µM/L and incubation carried out at 37 °C for 20 minutes.

Sperm DNA Fragmentation Index (DFI) Assessment

Sperm DNA fragmentation was assessed using the Sperm Chromatin Structure Assay (SCSA) according to the manufacturer's instructions (DFI Kit, BKR802, Wuhan Biocarry Technology Co., Ltd.). Briefly, semen samples were diluted to a concentration of 1–2 × 10^6/mL with the provided buffer (Solution A). Subsequently, 100 µL of acidic denaturation solution (Solution B) was added to 400 µL of the sperm suspension for 30 seconds. The reaction was then stopped by adding 1.2 mL of acridine orange (AO) staining solution (Solution C). AO is a metachromatic dye that emits green fluorescence when intercalated into double-stranded DNA and red fluorescence when associated with single-stranded DNA. The stained samples were immediately analyzed using a flow cytometer, and a minimum of 5,000 events per sample were recorded. The DFI was calculated as the ratio of red fluorescence (indicating denatured, fragmented DNA) to total (red plus green) fluorescence.

Extraction of DNA

Semen samples were processed for DNA extraction using the DP304 Cell Genome Extraction Kit (Cellpro, Beijing TIANGEN) in accordance with the manufacturer's instructions. The lysis buffer was supplemented with 1 M DTT to facilitate efficient digestion of the highly condensed chromatin structure in the sperm head.

Sperm MDB Detection [14]

The DNA concentration was adjusted to 10 ng/µL. A reaction mixture was prepared by combining 2 µL of 10× TdT buffer, 2 µL of dATP (Sangon Biotech), 8.5 µL of sterile deionized water, and 0.5 µL of TdT enzyme (NEB) with the sperm DNA solution. The mixture was incubated at 37 °C for 60 minutes, followed by 20 minutes at 75 °C. Subsequently, 5 µL of SD probes (400 nM) were added, and detection was performed using a qPCR instrument under the following conditions: 10 cycles of 30 seconds each at 37 °C. (The SD

probe sequence is provided in Table S1.)

Procedure of Transmission Electron Microscopy (TEM)

For detailed operational procedures, please refer to the supplementary material entitled "Protocol for Ultrastructural Analysis of Sperm Using Transmission Electron Microscopy (TEM)" based on the Hitachi HT7800 TEM system.

Procedure of Scanning Electron Microscopy (SEM)

For detailed methodological protocols, please see the supplementary document titled "Protocol for Sperm Ultrastructural Examination via Scanning Electron Microscopy (SEM)" using the Hitachi SU8100 system.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). All statistical analyses were conducted using SPSS software (version 22; IBM Corp.). Normality of data distribution was verified using the Shapiro–Wilk test, and homogeneity of variances was assessed with Levene's test, given the relatively small sample size. Comparisons of sperm parameters before and after the Swim-Up procedure were performed using paired t-tests. Given the individual variability in sperm DNA fragmentation (SDF) levels, a paired analysis approach was employed to more accurately evaluate the effects of PVP exposure. $P < 0.05$ was considered statistically significant.

Results

Sperm parameters

This study evaluated 98 semen samples comprising 56 asthenozoospermic patients (progressive motility <32% per WHO 6th edition criteria) and 42 normozoospermic controls (progressive motility ≥32%, normal morphology ≥4%). Demographic parameters (**Table S2**) were comparable between groups (age: 31.1 ± 5.4 vs 33.5 ± 4.2 years, p=0.32; abstinence period: 3.6 ± 1.3 vs 3.5 ± 1.1

days, p=0.41). Swim-up processing significantly enhanced sperm quality in both cohorts, with progressive motility increasing from 20.3 ± 5.0% to 43.5 ± 10.6% in asthenozoospermic samples and from 49.7 ± 8.9% to 69.8 ± 6.3% in controls (both $p<0.001$). These results demonstrate the swim-up method's efficacy for sperm quality enrichment across different semen parameters, thereby providing standardized preparations for subsequent PVP exposure experiments.

Detection principle and validation of principles

In **Fig. 1A**, sperm DNA breakage generates 3'-OH groups at the breakpoints. These 3'-OH groups are directly recognized by Terminal deoxynucleotidyl transferase (TdT). In the polymerization solution, free dATP extends a poly-A sequence from the 3'-OH ends. The poly-A sequence is identified in the "toe" region of the Strand Displacement Probe (SD Probe), which consists of an 18-base short chain with a FAM fluorophore and a 28-base long chain with a BHQ-1 fluorescence quencher. This recognition leads to separation of the short chain from the long chain, causing detachment of the FAM fluorophore from the BHQ-1 quencher and resulting in a fluorescent signal. The fluorescence signal intensity correlates with the number of 3'-OH groups[14]. To precisely quantify the number of breakpoints (i.e., 3'-OH groups), we utilized a DNA strand (S001) with a 3'-OH end as a standard (**Fig. 1B**).

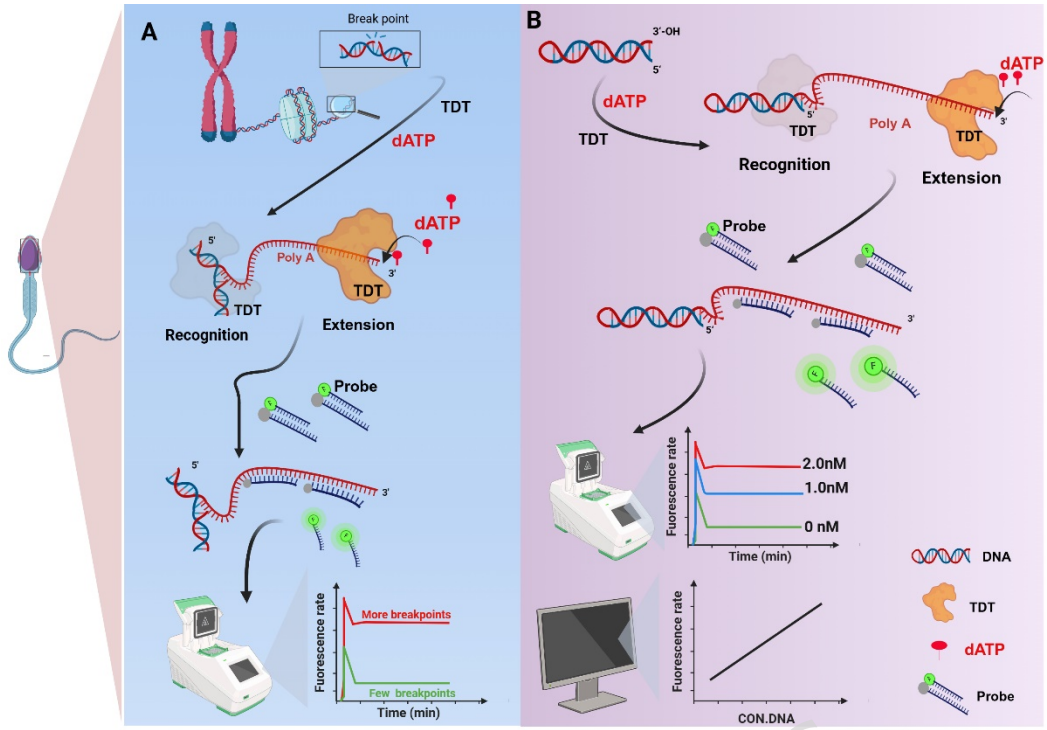

Fig.1 Schematic diagram of detection principle

A: Schematic diagram illustrating TdT enzyme coupled with displacement probe to detect DNA breakpoints and generate fluorescent signals. B: Schematic diagram illustrating the construction of a fitting curve for the number of 3'-OH groups and fluorescence signals using a standard sample. (Created with BioRender.com)

Using TdT, we identified the 3'-OH groups, and in the polymerization solution, free dATP extended a poly-A sequence from the 3'-OH ends. Subsequently, a displacement probe was employed to detect the poly-A sequence, resulting in fluorescence emission. By establishing a fitting equation based on the number of 3'-OH groups and fluorescence values obtained from the standard, we accurately determined the number of 3'-OH groups in sperm DNA.

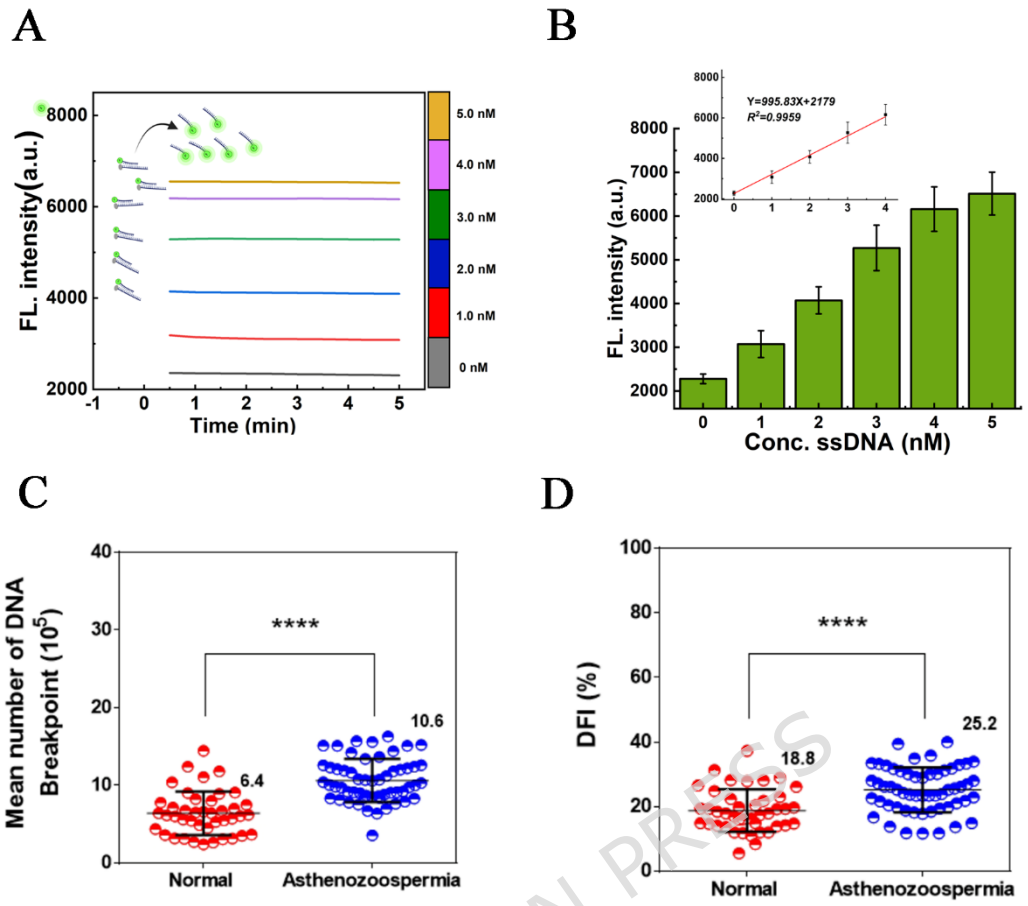

Fig. 2 Effects of PVP exposure on sperm DNA damage (MDB) in normozoospermic (n=42) and asthenozoospermic (n=56) samples. A: Representative fluorescence signal profiles of serially diluted standard samples (S001) in the detection system; B: Standard calibration curves showing linear regression analysis between fluorescence intensity and 3'-OH group concentration; C: Baseline sperm DNA breakpoints (MDB, $\times 10^5$) in unprocessed samples: normozoospermic (6.4 ± 2.8) vs asthenozoospermic (10.6 ± 2.7; ****p<0.0001). (D) DNA fragmentation index (DFI, %) in raw semen: normozoospermic (18.8 ± 6.6%) vs asthenozoospermic (25.2 ± 6.9 %; ****p<0.0001).

This study employed a gradient series of S001 standards (0, 10, 20, 30, 40, and 50 nM in 20 μL reaction system) to validate the quantitative capacity of the detection system for 3'-OH termini and establish the standard curve (**Fig. 2A**). The limit of detection (LOD) was determined to be 0.05 nM using the signal-to-noise ratio method (S/N=3), demonstrating the high sensitivity of this assay. As shown

in **Fig. 2B**, the detection system exhibited excellent linearity (R^2=0.9959) within the 0-4.0 nM concentration range, enabling the establishment of a linear regression equation between the quantity of 3'-OH termini and fluorescence signal intensity. Based on this standard curve, the precise quantification of 3'-OH termini corresponding to DNA breakpoints in test sperm DNA samples can be achieved by measuring their fluorescence signals.

We have improved the DNA extraction method and successfully extracted sperm DNA with good purity and concentration (see **Fig.S1**). In this detection system, the sperm DNA sample was loaded with 20 ng. Based on an average of 15 billion base pairs per human sperm cell, with each base pair having an average mass of 660, this sample corresponds to approximately 12, 000 sperm DNA strands. Assuming mean number of breakpoints per sperm DNA strand (n), as determined in the 20 μL detection system corresponding to 0.1 nM of 3'-OH groups, we calculated 'n' to be 1.98×10^5, indicating MDB as 1.98×10^5. Detailed is described in **Supplemental Calculation Methods**.

Mean number of DNA breakpoints

To validate the reliability of our MDB detection method, we conducted inter-operator reproducibility tests and spike-recovery experiments. For reproducibility assessment, three independent operators analyzed the same set of samples (n=15), showing excellent consistency with mean relative standard deviation (RSD) <5% (**Table S3**).

For accuracy evaluation, we performed spike-recovery tests using 15 sperm DNA samples (**Table S4**). After measuring baseline MDB values (MDB_1), each sample was spiked with 0.1 nM synthetic ssDNA standard and re-measured (MDB_2). Recovery rates calculated as [(MDB_2-MDB_1)/0.1 nM] ×100% ranged from 88.3% to 107.5%, with

a mean of 97.62 % ± 4.15 %, demonstrating good accuracy. These results confirm the method's reliability for quantitative sperm DNA breakpoint analysis. Following comprehensive validation of our MDB detection method, we evaluated sperm DNA breakpoints in 56 asthenozoospermic patients and 42 normozoospermic controls (**Table S5**). The results demonstrated that asthenozoospermic samples exhibited significantly higher baseline MDB values [(10.6 ± 2.7) × 10^5] compared to normozoospermic controls [(6.4 ± 2.8) × 10^5; $p < 0.0001$, independent t-test; Fig. 2C]. Similarly, DNA fragmentation index (DFI) analysis revealed elevated levels in the asthenozoospermic group [(25.2 ± 6.9) %] relative to controls [(18.8 ± 6.6) %; $p < 0.0001$; Fig. 2D]. Furthermore, ROC curve analysis demonstrated superior diagnostic performance of the MDB metric, with an area under the curve (AUC = 0.8695, 95% CI: 0.7931–0.9459) significantly higher than that of DFI (AUC = 0.7628, 95% CI: 0.6543–0.8512; Fig.S2).

Further assessment of post-swim-up sperm samples showed that normozoospermic specimens maintained significantly lower MDB levels (2.8 ± 1.5) × 10^5 than asthenozoospermic samples (6.7 ± 1.4) × 10^5; $p < 0.001$). Consistent with this trend, DFI measurements in post-swim-up samples were also markedly reduced in normozoospermic sperm (10.6 ± 4.3) % compared to asthenozoospermic sperm (17.5 ± 5.1) %; $p < 0.001$). These findings collectively suggest an association between asthenozoospermia and elevated sperm DNA damage levels, as measured by both MDB and DFI assays, which persists even following sperm selection through swim-up processing.

Sperm samples from 56 asthenozoospermic patients and 42 normozoospermic controls were processed through swim-up techniques, followed by co-incubation with 10% polyvinylpyrrolidone

(PVP) solution under strictly controlled conditions: 37°C in 5% CO_2 atmosphere for predetermined durations (5, 10, 20, and 30 minutes). This study employed a novel probe technology to quantitatively assess the effects of varying PVP exposure durations on sperm DNA breakpoints (MDB). Throughout the 30-minute observation period, MDB levels in the PVP-free control groups remained stable (normozoospermic: 2.81-2.96 $\times 10^5$; asthenozoospermic: 7.50-7.86 $\times 10^5$), indicating that the culture medium components, specific operational procedures, and observation duration employed in the experiment did not significantly affect sperm DNA integrity. Against this background, when compared with the corresponding PVP-free control groups at each time point, the PVP-treated groups exhibited time-dependent damaging effects. As shown in Fig. 3A, in normozoospermic samples, 5-minute exposure did not cause a significant increase in MDB ($p=0.33$), whereas 10-minute exposure led to a significant elevation ($p=0.025$), peaking at 30 minutes (198% of control). Asthenozoospermic samples demonstrated a similar pattern (Fig. 3B): the 5-minute group showed no significant difference in MDB, a significant increase began at 10 minutes ($p=0.011$), and cumulative damage reached its maximum at 30 minutes (235% of control). These results suggest that the observed DNA damage is primarily associated with PVP exposure.

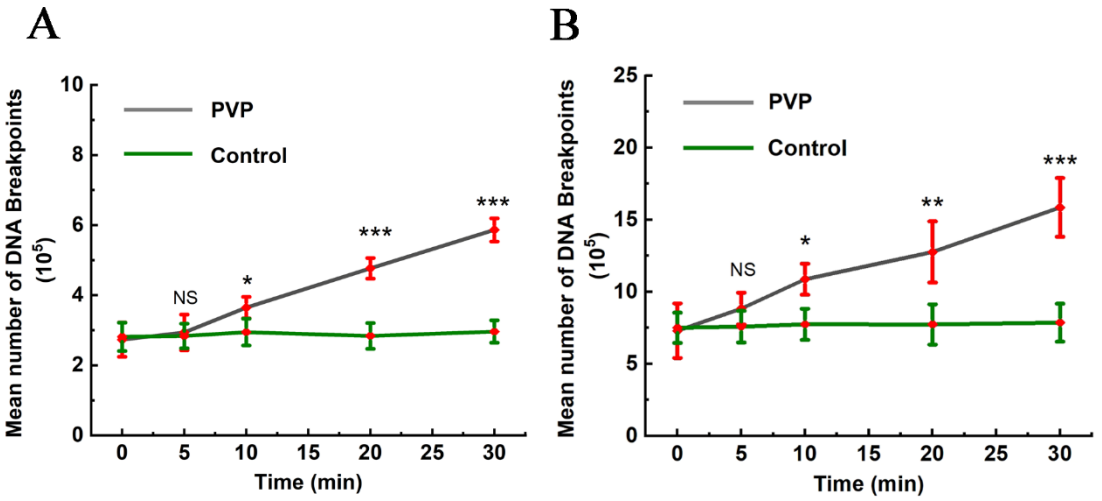

Fig. 3. Time-dependent effects of 10% PVP exposure on MDB counts in (A) normozoospermic samples (n=42) and (B) asthenozoospermic samples (n=56). Data are presented as mean ± SD compared to the corresponding PVP-free control at each time point. Statistical significance was determined by paired mixed-effects model (*p<0.05, **p<0.01, ***p<0.001 vs matched PVP-free control at the same time point); NS: not significant (p>0.05).

Although 10% PVP is commonly employed as the standard concentration in IVF laboratories, our study sought to systematically examine the dose-dependent effects of PVP on sperm DNA integrity. Using a standardized 10-minute incubation protocol, we evaluated four PVP concentrations (5%, 10%, 15%, and 20%) across 15 normozoospermic (Fig. 4A) and 15 asthenozoospermic samples (Fig. 4B). In normozoospermic samples, incubation with 5% PVP did not produce a statistically significant increase in DNA breakpoints ($p = 0.22$). In contrast, exposure to 10% PVP resulted in significant DNA damage ($p < 0.05$). A comparable pattern was observed in asthenozoospermic samples, where 5% PVP exposure showed no significant DNA damage, while 10% PVP demonstrated a pronounced damaging effect ($p < 0.01$). Collectively, these results suggest that 5% PVP may represent a safer alternative for maintaining DNA integrity under these experimental conditions, whereas PVP at concentrations of 10% and above appears to initiate

detectable DNA damage in both sample types.

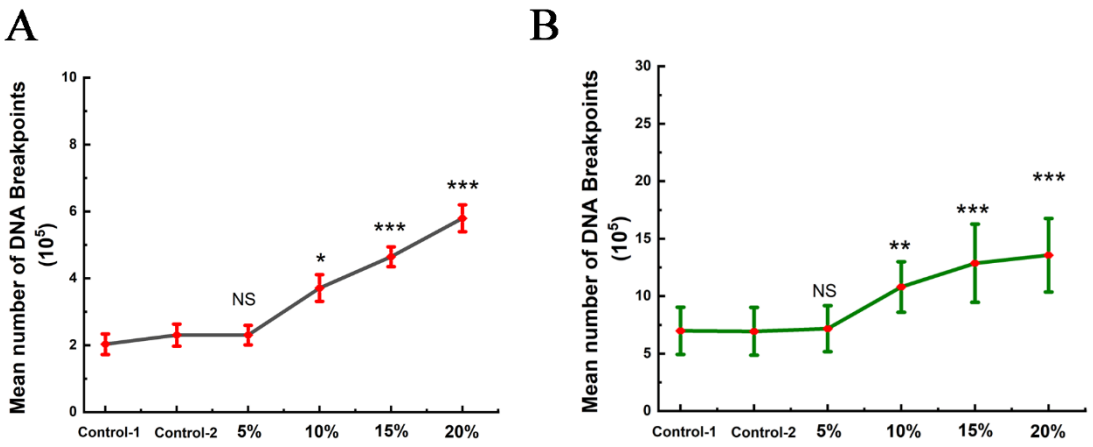

Fig.4. Dose-dependent effects of PVP exposure on sperm DNA fragmentation. (A) Normozoospermic (N=15) and (B) asthenozoospermic (N=15) samples were analyzed. Control-1: Baseline MDB (unincubated swim-up processed sperm); Control-2: Procedure control (sperm incubated in PVP-free medium). Paired t-tests compared each PVP concentration against matched Control-2 values from the same ejaculate. Data presented as mean ± SD (*p<0.05, **p<0.01, ***p<0.001).

Furthermore, reactive oxygen species (ROS) levels were assessed in 20 randomly selected samples following incubation with 10% PVP. As shown in Fig. S3, while no significant ROS changes were detected during the initial 5 minutes of exposure, a marked increase was observed after 10 minutes of incubation.

Microstructure changes

Exposure to PVP was associated with sperm DNA damage. To further investigate the structural alterations, we employed both scanning and transmission electron microscopy to examine sperm ultrastructure. Scanning electron microscopy revealed a series of morphological changes in the sperm plasma membrane, including structural modifications (Fig. 5A), significant swelling (Fig. 5B), membrane collapse (Fig. 5C), and overt damage (Fig. 5D). Transmission electron microscopy analysis demonstrated time-dependent ultrastructural alterations: acrosome swelling was observed after 5 minutes (Fig. 6A), progressed to more pronounced

acrosomal membrane swelling by 10 minutes (Fig. 6B), followed by disorganized and swollen mitochondrial arrangements in the midpiece after 20 minutes (Fig. 6C), and ultimately advanced to acrosomal membrane rupture after 30 minutes of exposure (Fig. 6D). These consistent ultrastructural changes were evident in both asthenozoospermic and normozoospermic samples.

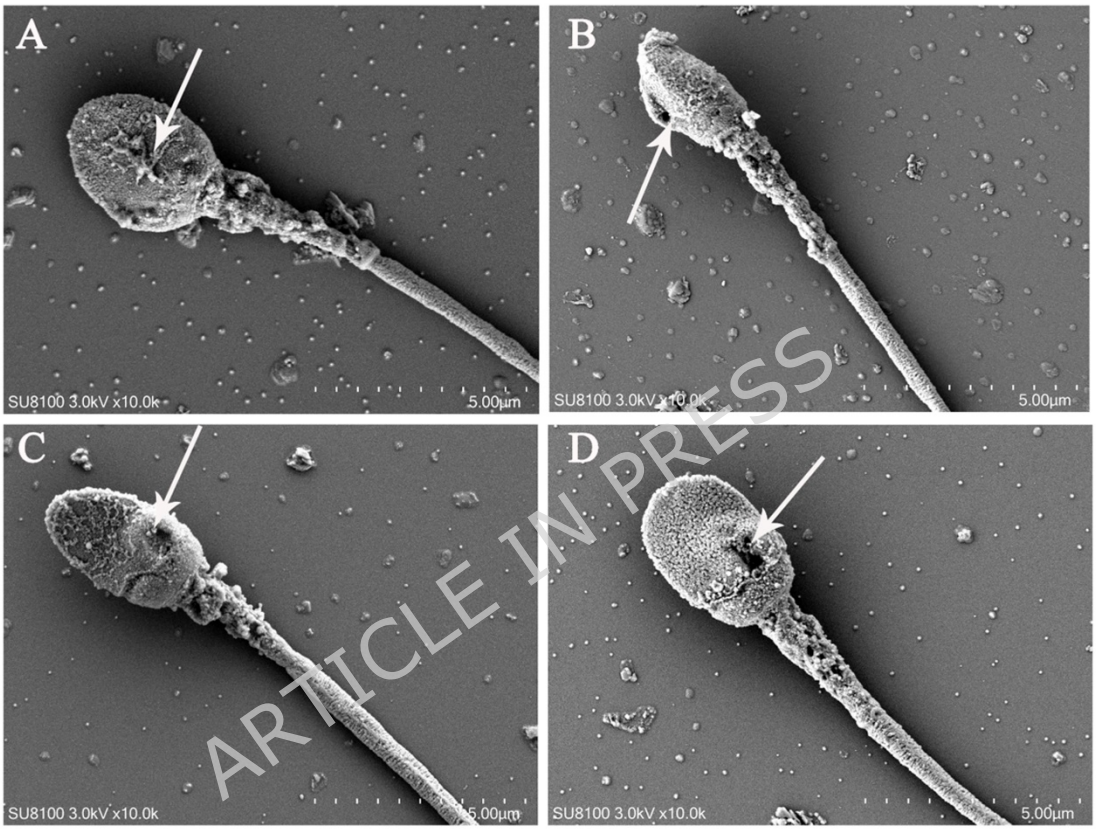

Fig. 5 The scanning electron microscopy observations of sperm surface microstructure at different treatment times: A (5 min group); B (10 min group); C (20 min group); D (30 min group). The white arrows indicate changes in the sperm plasma membrane microstructure.

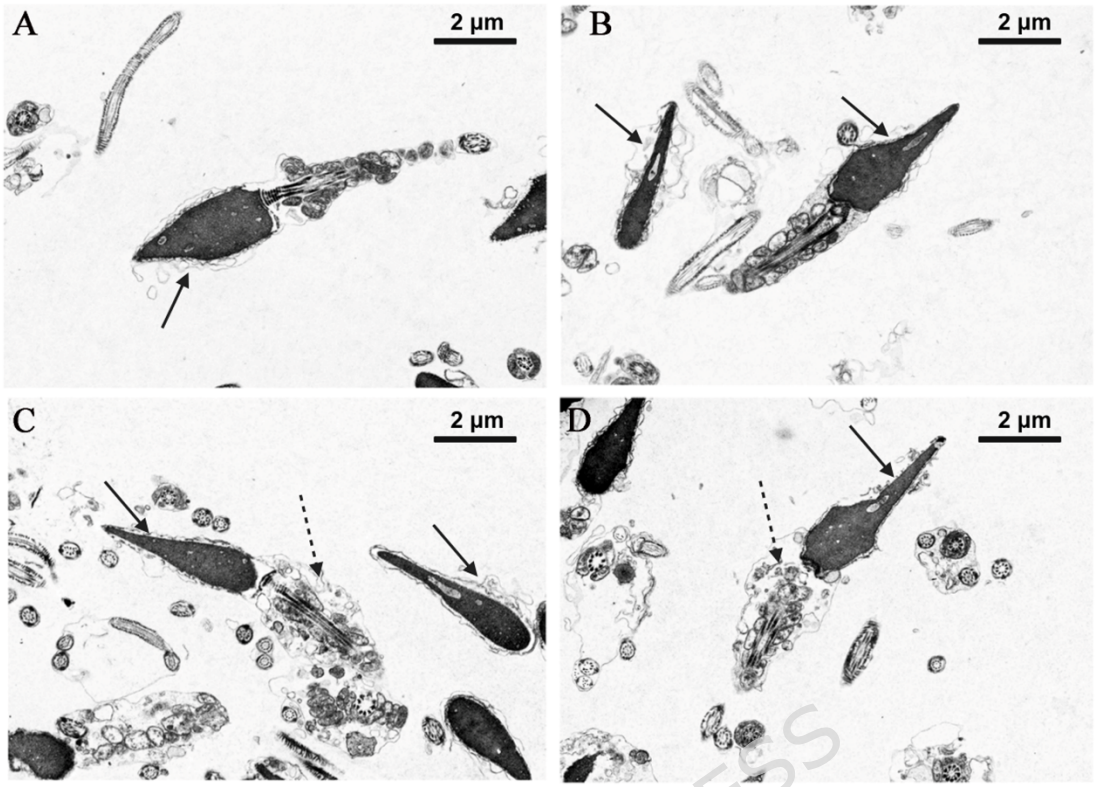

Fig. 6 Sperm internal microstructure under transmission electron microscopy at different treatment times: A (5 min group); B (10 min group); C (20 min group); D (30 min group). The black solid arrows indicate swelling of the acrosome membrane in sperm; the black dashed arrows indicate disordered and swollen arrangement of sperm mitochondrial.

Discussion

Polyvinylpyrrolidone (PVP) [10,15] is extensively utilized in assisted reproductive technology (ART) laboratories to immobilize sperm during intracytoplasmic sperm injection (ICSI). While its ability to facilitate sperm handling is well-established, growing evidence suggests that PVP may adversely affect sperm integrity—particularly at the molecular level. This study aimed to comprehensively evaluate the structural and DNA-level effects of PVP exposure on human spermatozoa from both normozoospermic and asthenozoospermic individuals, using high-resolution imaging and a novel high-throughput DNA breakpoint detection technique.

Our data suggest that short-term exposure (5 minutes) to 10% PVP does not cause significant DNA fragmentation, as quantified by the number of DNA breakpoints. However, ultrastructural alterations—particularly swelling and disruption of the acrosomal membrane—were already detectable via electron microscopy. After 10 minutes of exposure, a marked increase in DNA breakpoints was observed, accompanied by further deterioration of sperm head morphology. These results align with earlier reports by Nabi et al[10].and Sterler **[16]** et al, but extend them by providing quantitative, large-scale DNA breakpoint data and correlating them with submicroscopic anatomical changes.

The observed PVP-induced sperm damage may involve oxidative stress pathways. Our data are consistent with a mechanism whereby PVP exposure elevates intracellular reactive oxygen species (ROS) levels, potentially leading to sperm membrane lipid peroxidation and subsequent DNA strand breaks. These findings align with established literature demonstrating the association between ROS overproduction and sperm chromatin damage, including double-strand breaks. Previous investigations by Wang et al.[17] have indicated ROS involvement in sperm DNA damage, while subsequent research[18] has documented increased double-strand breaks under oxidative stress conditions. Similarly, Sayme et al.[19] a have highlighted how oxidative stress from excessive ROS generation can substantially impair sperm function and structural integrity.

Oocytes do possess robust DNA repair mechanisms (such as base excision repair and double-strand break repair pathways), which can partially correct sperm-derived DNA damage following fertilization[20,21]. However, existing evidence[22-24] indicates that this repair capacity varies significantly among individuals and is highly dependent on oocyte quality. Furthermore, when sperm DNA

damage (e.g., MDB values) exceeds a certain threshold, it may surpass the reparative capacity of the oocyte, leading to erroneous or incomplete repairs. This can subsequently result in embryonic developmental arrest (particularly during the blastulation stage) or increase the risk of paternal-origin chromosomal aneuploidy. Therefore, accurate assessment of sperm DNA damage remains crucial.

Notably, this study is the first to demonstrate a concentration- and time-dependent effect of PVP on both normal and pathological sperm samples using a highly sensitive DNA breakpoint assay capable of evaluating over 12,000 sperm per sample. This method offers considerable advantages over traditional approaches (e.g., SCD[25], SCSA[26], TUNEL[27]) in terms of quantitation, accuracy, and throughput, thereby providing a more reliable assessment of PVP-related genotoxicity.

An important finding of this study is the differential susceptibility to PVP between normozoospermic and asthenozoospermic samples. Asthenozoospermic sperm not only started with a higher baseline MDB but also exhibited a more pronounced relative increase in DNA breakpoints upon prolonged PVP exposure. This suggests that the inherent biological deficiencies in asthenozoospermic sperm, potentially including compromised DNA integrity and antioxidant defense mechanisms, render them more vulnerable to external stressors like PVP. Consequently, in clinical ICSI practice, especial vigilance regarding PVP exposure time is warranted when handling semen samples from patients with asthenozoospermia.

Based on these findings, we suggest minimizing PVP exposure time during ICSI procedures—preferably limiting to 5 minutes or less when using 10% PVP—and considering lower concentrations (e.g., 5%) where practicable. Additionally, our data support further

investigation of alternative sperm immobilizing agents. Previous studies on hyaluronic acid[28,29] and thymoquinone[30] have demonstrated potential for maintaining sperm motility while preserving DNA integrity, indicating that alternative approaches with improved safety profiles warrant consideration.

While this study provides valuable insights into PVP-induced sperm DNA damage, several limitations should be acknowledged. First, the in vitro experimental setting may not fully replicate the environment encountered during actual ICSI procedures. Second, although our sample size was sufficient to detect statistically significant effects, larger multicenter studies would strengthen the generalizability of these findings. Third, the clinical relevance of the observed DNA damage levels on embryo development and pregnancy outcomes remains to be established through prospective clinical trials. Finally, while we identified a correlation between PVP exposure and ROS elevation, the precise molecular mechanisms underlying PVP-induced oxidative stress require further investigation.

Based on the experimental evidence obtained in this study, we propose the following considerations for clinical practice: (1) 5% PVP concentration may be preferable for routine use ;(2) when conventional 10% PVP is employed in ICSI procedures, exposure duration should be carefully monitored and limited to 5 minutes or less.

From an operational perspective, implementing reduced PVP exposure times requires minimal procedural adjustments while offering potential biological advantages. The modified protocol does not require additional equipment or reagents, with the primary modification being the implementation of precise timing protocols—a measure that can be incorporated into existing quality management systems with minimal resource allocation. The potential benefits of reduced DNA fragmentation and improved

embryonic genetic quality could potentially contribute to enhanced clinical pregnancy rates and lower miscarriage risk, which may ultimately improve cost-effectiveness per live birth. While prospective clinical studies are needed to validate these outcomes, the risk-benefit profile appears favorable considering the modest implementation requirements.

This study provides valuable experimental evidence for optimizing sperm processing protocols in assisted reproductive technology, particularly through its systematic investigation of concentration-dependent PVP effects across both normozoospermic and asthenozoospermic samples. Future investigations could focus on developing optimized PVP treatment strategies tailored to different sperm pathological conditions. To support standardization of laboratory practices, reproductive centers may consider implementing standardized operating procedures that incorporate concentration verification and precise timing controls.

Conclusions

The application of polyvinylpyrrolidone (PVP) in assisted reproductive technology warrants attention to its potential reproductive toxicity. Our findings show that 5-minute exposure to PVP induces ultrastructural damage to the sperm acrosomal membrane, while 10-minute exposure significantly increases DNA breakpoints along with elevated reactive oxygen species levels. Concentration-dependent experiments demonstrate that 5% PVP is less damaging to sperm DNA integrity compared to conventional 10% concentrations under our experimental conditions. For ICSI procedures, we suggest: (1) limiting PVP exposure time to 5 minutes or less; (2) considering 5% PVP as a preferred concentration; (3) implementing standardized operating protocols.

Abbreviations

PVP Polyvinylpyrrolidone

SCD Sperm Chromatin Dispersion

OAT Oligoasthenoteratozoospermia

TUENL Terminal deoxynucleotidyl transferase-mediated dUTP Nick-End Labeling

SCSA Sperm Chromatin Structure Assay

TDT Terminal deoxynucleotidyl transferase

PR Progressive motility

MDB Mean number of DNA Breakpoint

ART Assisted Reproductive Technology

Supplementary Information

The online version contains supplementary material available at

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Author contributions

M.W conceived and designed the study; analyzed, and interpreted the data; H.W and F.Z prepared data interpretation, made revision to the paper; H.Y, F.Z and K.D generated and collected the data. Y.M, L.P and H.W analyzed, and interpreted the data; L.P, F.Z and B.Y prepared the final draft of the paper. All authors contributed to approve the final manuscript.

Data Availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

This project received approval from the Ethics Committee of the General Hospital of Ningxia Medical University (KYLL2022-0144) and was conducted following informed consent obtained from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare no potential conflicts of interest regarding the publication of this research.

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