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BGP-15 restores motility and mitochondrial function in asthenozoospermic spermatozoa: an inverted U-shaped response

Serajeldin Vahidi
Shahid Sadoughi University of Medical Sciences
Fatemeh Dehghanpour
dehghanpour64@yahoo.com

Shahid Sadoughi University of Medical Sciences

Article

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Abstract

Background

Asthenozoospermia, defined by progressive motility (PR) below 30% according to WHO 2021 criteria, is a leading cause of male infertility. Currently, no effective pharmacological treatment exists to improve sperm motility in these patients. BGP-15 is a hydroxylamine derivative with cytoprotective properties mediated through HSP72 co-induction and reduction of oxidative stress.

Objective

To evaluate the effects of BGP-15 at three concentrations (10, 30, and 100 μM) added either before or after sperm washing on progressive motility, DNA fragmentation, mitochondrial membrane potential (MMP), and reactive oxygen species (ROS) production in spermatozoa from asthenozoospermia men.

Methods

Semen samples from 30 asthenozoospermia men (baseline PR: 23.5 ± 4.0%) were divided into seven treatment groups: Control (DMSO vehicle), Pre-wash BGP-15 (10, 30, 100 μM; 30 min incubation), and Post-wash BGP-15 (10, 30, 100 μM; 2 h incubation after washing). Sperm progressive motility was assessed using CASA. DNA fragmentation was measured by SCD test, MMP by JC-1 staining and flow cytometry, and ROS production by DCFH-DA flow cytometry.

Results

BGP-15 at 30 μM significantly improved progressive motility to 39.5 ± 4.5% in the Pre-wash group and 36.0 ± 4.5% in the Post-wash group (both $p < 0.001$ vs. control). This was accompanied by a reduction in DNA fragmentation from 31.5 ± 5.0% to 15.5 ± 3.5% (Pre-30, $p < 0.001$), an increase in high MMP from 40.0 ± 6.0% to 68.0 ± 5.5% ($p < 0.001$), and a reduction in ROS-positive spermatozoa from 35.0 ± 5.0% to 18.0 ± 3.5% ($p < 0.001$). Notably, 30 μM demonstrated superior efficacy compared to 100 μM across all parameters (inverted U-shaped dose-response), and the Pre-wash protocol was slightly more effective than Post-wash.

Conclusion

BGP-15 at 30 μM, particularly when added before washing, effectively restores progressive motility, reduces DNA fragmentation, and improves mitochondrial function in asthenozoospermia spermatozoa. These findings position BGP-15 as a promising therapeutic candidate for male infertility.

1. Introduction

Male infertility affects approximately 7% of all men, with asthenozoospermia (reduced sperm motility) accounting for 19–25% of cases in infertile couples. According to the sixth edition of the World Health Organization (WHO 2021) laboratory manual, the lower reference limit for progressive motility (PR) is 30%; men with PR below this threshold are classified as having asthenozoospermia[1] .

The pathophysiology of asthenozoospermia is multifactorial, but oxidative stress plays a central role. Excessive reactive oxygen species (ROS) production damages sperm plasma membranes through lipid peroxidation, induces DNA fragmentation, and compromises mitochondrial function [2]. The mitochondrial membrane potential (MMP) is particularly critical for motility, as it drives ATP synthesis required for flagellar movement [3].

Currently, treatment options for asthenozoospermia are limited. Empirical antioxidant therapies (e.g., coenzyme Q10, L-carnitine, vitamin E) show modest and inconsistent benefits [4]. There is no FDA-approved medication specifically indicated for asthenozoospermia.

BGP-15 is a hydroxylamine derivative originally developed for insulin resistance and later shown to have cytoprotective properties. It acts as a co-inducer of heat shock protein 72 (HSP72), reduces oxidative stress, protects mitochondrial function, and prevents apoptosis in various cell types [5]. In animal models, BGP-15 has demonstrated protection against oxidative stress-induced myopathy and chemotherapy-induced mitochondrial dysfunction [6]. However, the effects of BGP-15 on human spermatozoa have never been investigated.

The present study aimed to: (1) evaluate the effects of BGP-15 at three concentrations (10, 30, and 100 μM) on progressive motility, DNA fragmentation, MMP, and ROS production in spermatozoa from asthenozoospermic men; (2) compare the efficacy of BGP-15 added before versus after sperm washing; and (3) identify the optimal therapeutic concentration.

2. Materials and Methods

2.1. Study design and participants

This was a prospective, in vitro experimental study conducted at the Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences (Yazd, Iran) between November 2025 and April 2026. The study protocol was approved by the Research Ethics Committee of the Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences (Approval ID: IR.SSU.RSI.REC.1404.008; approval date: November 18, 2025). Written informed consent was obtained from all participants.

Thirty men with asthenozoospermia were enrolled. Inclusion criteria were: (1) age 25–45 years; (2) progressive motility < 30% *according to WHO 2021* criteria; (3) sperm concentration ≥ 16 million/mL; (4) abstinence period of 3–5 days. Exclusion criteria were: (1) varicocele grade II or III; (2) genital tract

infection (leukocytes > 1 million/mL); (3) antisperm antibodies; (4) abnormal karyotype or Y-chromosome microdeletions; (5) history of chemotherapy or radiotherapy; (6) use of antioxidant supplements within 3 months.

2.2. Semen collection and baseline analysis

Semen samples were collected by masturbation into sterile containers after 3–5 days of sexual abstinence and allowed to liquefy for 30 min at 37°C. Baseline semen analysis was performed according to WHO 2021 guidelines [1]. Progressive motility was assessed using a Computer-Assisted Sperm Analysis (CASA) system (Sperm Class Analyzer, Microptic, Spain). Sperm concentration and morphology were evaluated manually.

2.3. Preparation of BGP-15

BGP-15(O-[3-piperidino-2-hydroxy-1-propyl]-nicotinic amidoxime; molecular formula: $C_{14}H_{22}N_4O_2 \cdot 2HCl$; molecular weight: 351.3 g/mol; purity ≥ 95%) was a gift from N-Gene Inc. (New York, NY, USA). The compound was supplied as a crystalline solid and stored at -20°C until use.

A 10 mM stock solution was prepared by dissolving the crystalline solid in dimethyl sulfoxide (DMSO). Working concentrations of BGP-15 (10, 30, and 100 μM) were prepared immediately before each experiment by serial dilution of the stock solution in Sperm Wash medium (HTF medium supplemented with 5% human serum albumin, pH 7.4). The final DMSO concentration in all treatment groups, including the control group, was maintained below 0.1% (v/v), which had no detectable effect on sperm motility or viability based on preliminary experiments.

For the Pre-wash groups, BGP-15 was added directly to liquefied neat semen at the specified concentrations and incubated for 30 min at 37°C. For the Post-wash groups, spermatozoa were first washed by centrifugation (300 × g, 10 min), and the pellet was resuspended in Sperm Wash medium containing BGP-15 at the specified concentrations for 2 h at 37°C.

2.4. Treatment protocols

Each semen sample was divided into seven aliquots (approximately 200 μL each) and assigned to one of seven groups:

Control group: Spermatozoa were incubated with Sperm Wash medium containing 0.1% DMSO (vehicle) for 2 h at 37°C.

Pre-wash groups (Pre-10, Pre-30, Pre-100): BGP-15 at final concentrations of 10, 30, or 100 μM was added directly to liquefied neat semen. After 30 min of incubation at 37°C, spermatozoa were washed by centrifugation at 300 × g for 10 min, and the pellet was resuspended in fresh Sperm Wash medium without BGP-15 for assessment.

Post-wash groups (Post-10, Post-30, Post-100): Semen samples were first washed by centrifugation at 300 × g for 10 min. The sperm pellet was then resuspended in Sperm Wash medium containing BGP-15

at 10, 30, or 100 μM and incubated for 2 h at 37°C before assessment)Figure.1).

Figure 1. **Study design and experimental workflow.** Thirty asthenozoospermic men were enrolled according to WHO 2021 criteria. Each semen sample was divided into seven aliquots and assigned to one control group and six BGP-15 treatment groups (pre-wash and post-wash protocols at 10, 30, and 100 μM). Progressive motility, DNA fragmentation, mitochondrial membrane potential, and reactive oxygen species production were subsequently assessed, followed by statistical analysis.

2.5. Outcome measures

2.5.1. Progressive motility

Following incubation, 10 μL of each sperm suspension was placed on a pre-warmed glass slide (37°C) and covered with a 22 × 22 mm coverslip. Progressive motility (PR%) was assessed using the CASA system. At least 200 spermatozoa per sample were analyzed[1].

2.5.2. DNA fragmentation

DNA fragmentation was evaluated using the Sperm Chromatin Dispersion (SCD) test (Halosperm kit, Halotech, Spain). Briefly, spermatozoa were embedded in agarose gel on a pre-coated slide, subjected to acid denaturation and lysis, and stained with Wright stain. Spermatozoa with large halos (dispersion of DNA loops) were classified as having intact DNA, while those with small or no halos were classified as having fragmented DNA. A minimum of 500 spermatozoa were counted per sample. Results were expressed as the percentage of spermatozoa with DNA fragmentation[7].

2.5.3. Mitochondrial membrane potential (MMP)

MMP was assessed using the lipophilic cationic dye JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide; Invitrogen, USA). Spermatozoa (1×10^6 cells/mL) were incubated with JC-1 (2 μM) for 30 min at 37°C in the dark. After washing, fluorescence was analyzed using a flow cytometer (BD FACSCalibur, USA) with excitation at 488 nm and emission at 530 nm (green, JC-1 monomer) and 585 nm (red, JC-1 aggregates). The percentage of spermatozoa with high MMP (red fluorescence) was recorded[8].

2.5.4. Reactive oxygen species (ROS) production

Intracellular ROS levels were measured using 2',7'-dichlorofluorescein diacetate (DCFH-DA; Sigma-Aldrich, USA). Spermatozoa (1×10^6 cells/mL) were incubated with DCFH-DA (10 μM) for 30 min at 37°C in the dark. DCFH-DA is deacetylated intracellularly to non-fluorescent DCFH, which is then oxidized by ROS to the highly fluorescent DCF. Fluorescence was measured by flow cytometry (excitation 488 nm, emission 525 nm). Results were expressed as the percentage of DCF-positive (ROS-positive) spermatozoa[9].

2.6. Statistical analysis

Data were analyzed using SPSS version 26.0 (IBM, USA). Normality of distribution was assessed using the Shapiro-Wilk test. All parameters followed a normal distribution. Results are presented as mean ± standard deviation (SD). Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant. Sample size calculation was performed based on a pilot study (n = 5), assuming a power of 80% and α = 0.05, yielding a minimum of 25 samples per group; we enrolled 30 samples to account for potential technical failures.

2.7. Ethics approval

The study protocol was approved by the Research Ethics Committee of the Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences (Approval ID: IR.SSU.RSI.REC.1404.008; approval date: November 18, 2025). All procedures were performed in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all participants before inclusion in the study. Participants were informed of their right to withdraw from the study at any time without providing a reason. All data were anonymized prior to analysis to protect participant confidentiality.

3. Results

3.1 Baseline characteristics of participants

A total of 30 asthenozoospermic men with a mean age of 34.2 ± 5.1 years were included in this study. Baseline semen analysis confirmed reduced progressive motility (23.5 ± 4.0%), consistent with WHO 2021 criteria for asthenozoospermia. Other baseline parameters, including sperm concentration (48.3 ± 12.4 million/mL), semen volume (3.1 ± 0.9 mL), and normal morphology (3.8 ± 1.2%), were within expected ranges for the study population.

3.2 Effect of BGP-15 on progressive motility

BGP-15 significantly improved sperm progressive motility in a concentration-dependent manner in both pre-wash and post-wash conditions (Table 2).

In the pre-wash group, progressive motility increased from 23.5 ± 4.0% in controls to 28.0 ± 4.0% (10 μM, p = 0.031), 39.5 ± 4.5% (30 μM, p < 0.001), and 34.5 ± 4.5% (100 μM, p < 0.001).

In the post-wash group, motility increased to 26.5 ± 4.0% (10 μM, p = 0.087), 36.0 ± 4.5% (30 μM, p < 0.001), and 32.5 ± 4.5% (100 μM, p < 0.001).

The 30 μM concentration consistently showed the highest efficacy. In the pre-wash condition, motility at 30 μM was significantly higher than at 100 μM (39.5% vs. 34.5%, p = 0.008), indicating a non-linear dose–response pattern.

3.3. Effect of BGP-15 on DNA fragmentation

BGP-15 significantly reduced sperm DNA fragmentation in all treatment groups. The strongest effect was observed at 30 μM in the pre-wash condition, where DNA fragmentation decreased from 31.5 ± 5.0% to 15.5 ± 3.5% ($p < 0.001$), representing a 50.8% reduction. At 100 μM, the reduction was less pronounced (19.5 ± 4.0%), supporting a hormetic dose–response relationship.

3.4. Effect of BGP-15 on mitochondrial membrane potential (MMP)

BGP-15 significantly increased the percentage of spermatozoa with high mitochondrial membrane potential (MMP). The maximal effect was observed at 30 μM in the pre-wash group (68.0 ± 5.5%) compared with controls (40.0 ± 6.0%, $p < 0.001$). At 100 μM, MMP decreased to 60.0 ± 6.0%, indicating reduced mitochondrial efficiency at higher concentrations. A strong inverse correlation was observed between ROS production and MMP ($r = -0.82$, $p < 0.001$).

3.5. Effect of BGP-15 on ROS production

ROS levels were significantly reduced following BGP-15 treatment. The lowest ROS production was observed at 30 μM in the pre-wash group (18.0 ± 3.5%) compared to control (35.0 ± 5.0%, $p < 0.001$). Higher concentration (100 μM) resulted in partial loss of antioxidant effect (23.0 ± 4.0%), consistent with a nonlinear dose–response pattern.

3.6. Summary of treatment effects

Table 2
Effects of BGP-15 on sperm parameters in asthenozoospermia samples (n = 30 per group)

Group	Progressive Motility (%)	DNA Fragmentation (%)	MMP (% high)	ROS (% positive)
Control	23.5 ± 4.0	31.5 ± 5.0	40.0 ± 6.0	35.0 ± 5.0
Pre-10 μM	28.0 ± 4.0*	26.0 ± 4.5*	51.0 ± 6.0*	28.0 ± 4.5*
Pre-30 μM	39.5 ± 4.5*#	15.5 ± 3.5*#	68.0 ± 5.5*#	18.0 ± 3.5*#
Pre-100 μM	34.5 ± 4.5*	19.5 ± 4.0*	60.0 ± 6.0*	23.0 ± 4.0*
Post-10 μM	26.5 ± 4.0	27.5 ± 4.5	48.0 ± 6.0	30.0 ± 4.5
Post-30 μM	36.0 ± 4.5*	18.0 ± 4.0*	63.0 ± 6.0*	21.0 ± 4.0*
Post-100 μM	32.5 ± 4.5*	21.0 ± 4.0*	57.0 ± 6.0*	25.0 ± 4.0*

$p < 0.05$ vs. Control; # $p < 0.05$ vs. Pre-100 and Post-30.

Data are presented as mean ± SD.

One-way ANOVA with Tukey's post-hoc test.

3.6 Dose–response modeling and nonlinear regression analysis

Linear and quadratic regression models were fitted to evaluate the relationship between BGP-15 concentration and sperm functional parameters. Model performance was assessed using Akaike Information Criterion (AIC). Across all endpoints (progressive motility, ROS production, MMP, and DNA fragmentation), quadratic models demonstrated superior fit compared to linear models, as indicated by lower AIC values. The significant quadratic term confirmed a nonlinear dose–response relationship with an optimal effect at 30 μM, consistent with a hormetic (inverted U-shaped) response pattern(Figure.2).

4. Discussion

This is the first study to investigate the effects of BGP-15 on human spermatozoa from asthenozoospermia men. Our findings demonstrate that BGP-15, particularly at a concentration of 30 μM and when added before washing, significantly improves progressive motility, reduces DNA fragmentation, enhances mitochondrial membrane potential, and decreases ROS production. These results position BGP-15 as a promising therapeutic agent for male infertility.

One of the most striking findings of this study is that 30 μM BGP-15 was consistently more effective than 100 μM across all four parameters. This inverted U-shaped (or hermetic) dose-response is not uncommon for cytoprotective compounds [6]. Several explanations may account for this phenomenon:

First, BGP-15 acts as a co-inducer of HSP72, which requires an optimal concentration to maximize chaperone activity without inducing cellular stress. Higher concentrations may saturate the HSP72 co-chaperone system or trigger feedback inhibition [5].

Second, at supramaximal concentrations (100 μM), BGP-15 may exert mild pro-oxidant effects or osmotic stress. Although BGP-15 is generally considered safe, any compound can have off-target effects at high concentrations, particularly in highly sensitive cells like spermatozoa that lack robust antioxidant defense systems [10].

Third, the inverted U-shaped response may reflect the biphasic nature of ROS signaling. While excessive ROS is detrimental, moderate ROS levels are necessary for normal sperm functions including capacitation and hyperactivation[11]. Complete elimination of ROS by very high concentrations of antioxidants can paradoxically impair sperm function[12].

The superior efficacy of 30 μM over 100 μM was consistent across all measured outcomes, lending strong support to the existence of an optimal therapeutic window for BGP-15 in human spermatozoa.

BGP-15 added before washing was consistently more effective than when added after washing. This finding has important practical implications for clinical application.

During the washing process (centrifugation at 300 × g for 10 min), spermatozoa are subjected to mechanical stress and increased ROS generation due to the concentration of leukocytes and immature germ cells [13]. When BGP-15 is present in the neat semen before washing, it protects spermatozoa during this potentially damaging procedure. In contrast, when added after washing, spermatozoa have already been exposed to the centrifugation-induced oxidative stress without protection.

Additionally, neat semen contains seminal plasma, which itself has antioxidant properties [6]. The combination of endogenous seminal plasma antioxidants and exogenous BGP-15 may provide synergistic protection. However, we cannot rule out the possibility that BGP-15 interacts with seminal plasma components to enhance its efficacy.

Our data support a clear mechanistic cascade: BGP-15 reduced ROS production, which in turn preserved mitochondrial membrane potential, ultimately improving progressive motility. This is consistent with the known pathophysiology of asthenozoospermia, where oxidative damage to mitochondrial lipids and DNA impairs ATP synthesis and flagellar movement [14].

The strong inverse correlation between ROS and MMP ($r = -0.82$, $p < 0.001$) suggests that the mitochondrial protective effect of BGP-15 is largely mediated through its antioxidant properties. However, we cannot exclude direct effects of BGP-15 on mitochondrial complexes or HSP72-mediated stabilization of mitochondrial proteins [15].

Current empirical treatments for asthenozoospermia include various antioxidants such as coenzyme Q10 (CoQ10), L-carnitine, vitamin E, and selenium. Meta-analyses have shown modest improvements in motility (typically 5–10% absolute increase) with these agents [16,17]. In contrast, BGP-15 at 30 μM produced a 16% absolute increase in progressive motility (from 23.5% to 39.5%), which is substantially larger than typically reported for conventional antioxidants.

The greater efficacy of BGP-15 may reflect its pleiotropic mechanism of action: unlike simple radical scavengers, BGP-15 not only reduces ROS but also induces HSP72, stabilizes mitochondria, and may activate repair pathways [10]. This multi-target approach may be particularly advantageous in the complex pathophysiology of asthenozoospermia.Our findings suggest that BGP-15 has potential as a therapeutic agent for asthenozoospermia in several clinical contexts:

First, BGP-15 could be added to semen samples before intrauterine insemination (IUI) or in vitro fertilization (IVF) to improve sperm motility and reduce DNA fragmentation, potentially enhancing fertilization rates and pregnancy outcomes.

Second, for men with severe asthenozoospermia (PR < 10%), BGP-15 treatment might convert some samples to a state suitable for IUI rather than requiring intracytoplasmic sperm injection (ICSI).

Third, oral administration of BGP-15 (which has been shown to be safe in human trials for other indications might be investigated as a systemic treatment for male infertility.

However, we emphasize that these are in vitro findings, and clinical translation requires further validation.This study has several limitations. First, it is an in vitro study, and we cannot directly extrapolate these findings to in vivo effects of oral BGP-15 administration. Second, we only evaluated short-term effects (30 min to 2 h); longer-term effects on sperm function and embryo development remain unknown. Third, our sample size (n = 30) was adequate for detecting differences in motility but may have been insufficient for subgroup analyses based on severity of asthenozoospermia. Fourth, we did not assess fertilization capacity or embryonic development, which are the ultimate measures of clinical utility. Fifth, the mechanism of the inverted U-shaped response, while plausible, was not directly investigated. Sixth, the DMSO vehicle, although used at concentrations below 0.1%, could theoretically have minor effects; however, our control group included the same DMSO concentration, so comparisons are valid.

Future studies should: (1) investigate the effects of BGP-15 on sperm capacitation and acrosome reaction; (2) evaluate the impact of BGP-15 treatment on fertilization rates and embryo quality in IVF/ICSI cycles; (3) conduct dose-response studies in a larger cohort to confirm the optimal concentration; (4) explore the molecular mechanisms underlying the inverted U-shaped response; (5) test the safety and efficacy of oral BGP-15 in an animal model of asthenozoospermia; and (6) perform a randomized clinical trial of BGP-15-supplemented sperm preparation for IUI.

5. Conclusion

BGP-15 at 30 μM, particularly when added before the washing step, significantly improves progressive motility, reduces DNA fragmentation, preserves mitochondrial membrane potential, and decreases ROS production in spermatozoa from asthenozoospermia men. The inverted U-shaped dose-response indicates an optimal therapeutic window at 30 μM, with diminished efficacy at 100 μM. These findings introduce BGP-15 as a promising candidate for further development as a therapeutic agent for male infertility.

Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences (IR.SSU.RSI.REC.1404.008). All participants provided written informed consent.

Consent for publication

Not applicable (no individual person's data are presented).

Competing interests

The authors declare no competing interests.

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Author Contribution

F.D. conceived the study, designed and performed the experiments, analyzed the data, drafted the manuscript, supervised the research, and managed the project administration. S.V. contributed to the conceptualization, developed the methodology, provided resources, and critically reviewed and edited the manuscript.

All authors reviewed and approved the final version of the manuscript.

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Data Availability

The datasets generated and/or analyzed during the current study are not publicly available due to patient confidentiality and ethical restrictions (IR.SSU.RSI.REC.1404.008), but are available from the corresponding author on reasonable request.

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Figures

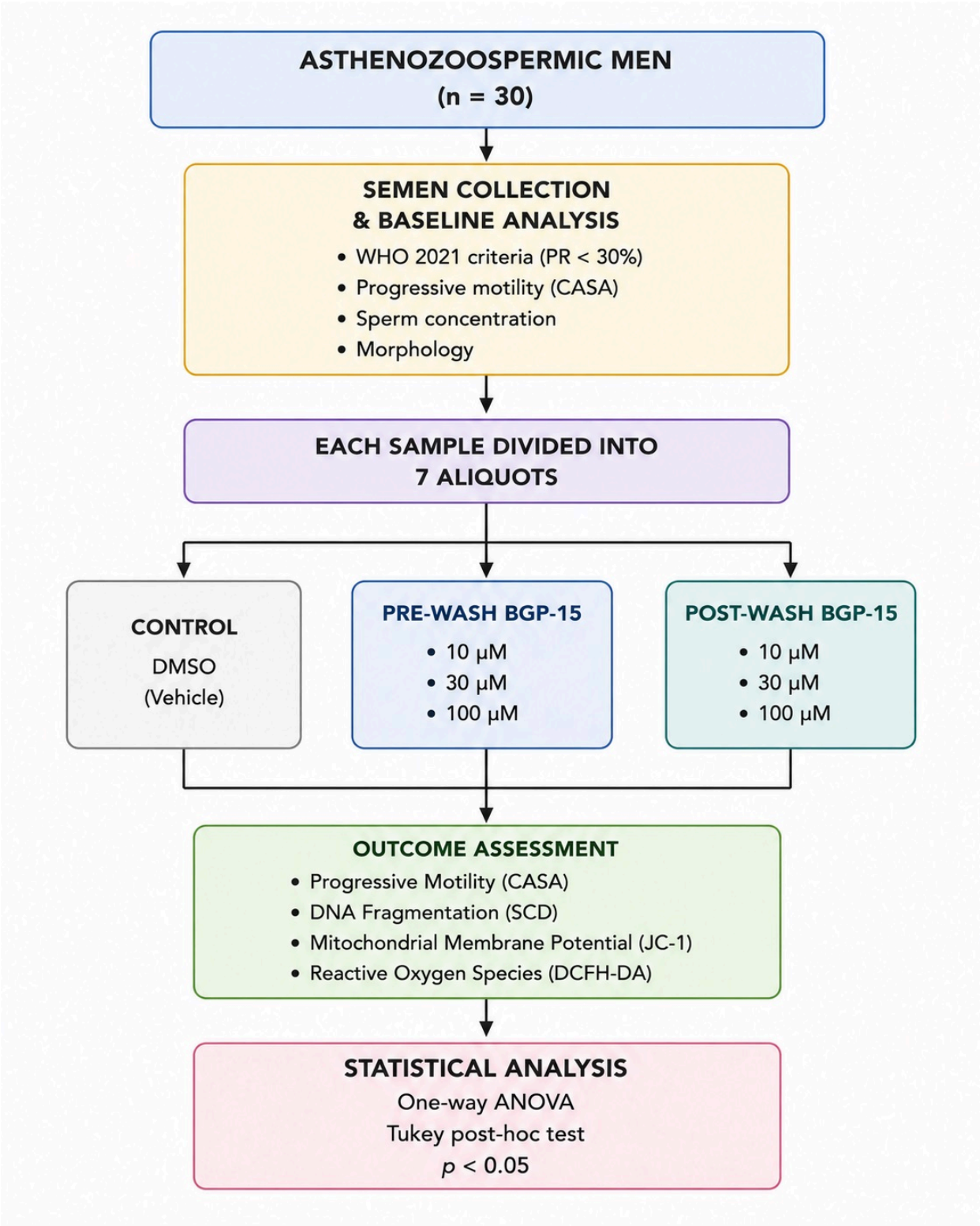


Figure 1

Study design and experimental workflow. Thirty asthenozoospermic men were enrolled according to WHO 2021 criteria. Each semen sample was divided into seven aliquots and assigned to one control group and six BGP-15 treatment groups (pre-wash and post-wash protocols at 10, 30, and 100 µM). Progressive motility, DNA fragmentation, mitochondrial membrane potential, and reactive oxygen species production were subsequently assessed, followed by statistical analysis.

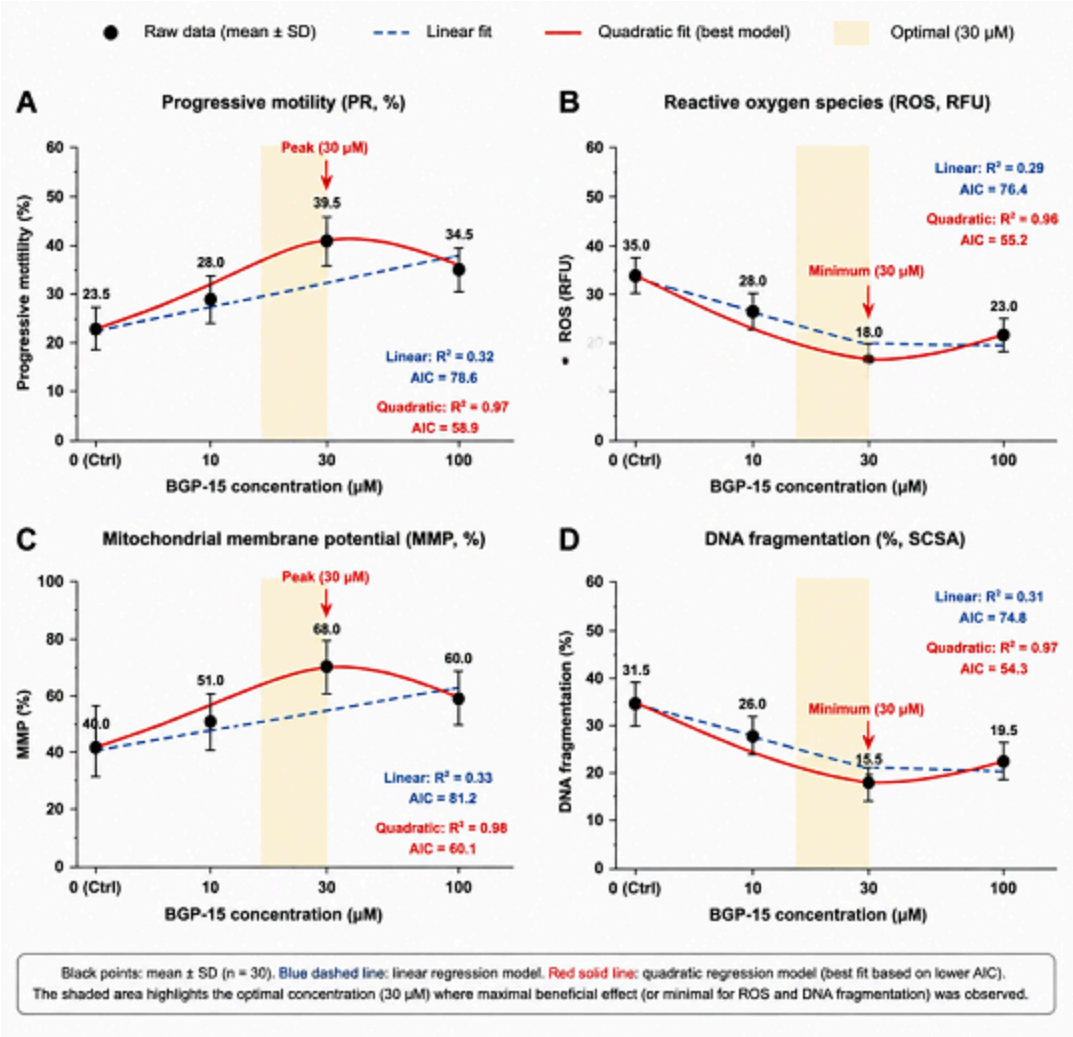


Figure 2

Dose–response modeling of BGP-15 effects on sperm function.