

Preliminary evaluation of NMN reprotoxicity using human sperm assay

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Short Report

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Abstract

Human spermatozoa are highly susceptible to oxidative injury owing to their polyunsaturated fatty acid-rich plasma membrane and limited antioxidant defenses. Nicotinamide mononucleotide (NMN), a key NAD⁺ precursor, has gained attention for its roles in cellular energetics and redox balance. However, direct evidence regarding its potential reprotoxic effects on human spermatozoa is lacking. This preliminary Human Sperm Assay (HSA) evaluated the compatibility of NMN with human sperm progressive motility. Seven normozoospermic semen samples were exposed to NMN at 0 (control), 50, 100, or 150 μ M. Progressive motility was assessed according to WHO 2021 criteria at baseline and after 0.5, 1, 2, 4, and 24 hours of incubation. NMN at 50 μ M and 100 μ M did not impair progressive motility relative to untreated controls throughout the 24-hour incubation. In contrast, exposure to 150 μ M NMN resulted in a substantial reduction in progressive motility. These preliminary findings suggest that NMN does not exert reprotoxic effects on sperm progressive motility at 50 and 100 μ M, whereas 150 μ M induces motility impairment. Larger studies with additional sperm function parameters are warranted to confirm the short-term safety of these lower concentrations.

Introduction

Oxidative stress is a major contributor to sperm dysfunction and male reproductive impairment. Human spermatozoa are particularly susceptible to oxidative injury because their plasma membrane is enriched with polyunsaturated fatty acids, while their endogenous antioxidant defenses are limited (Aitken et al., 2015). Even modest elevations in reactive oxygen species can impair sperm motility, membrane integrity, and fertilizing capacity (Agarwal et al., 2003). This intrinsic vulnerability highlights the importance of carefully evaluating the safety of candidate antioxidant compounds before their application in reproductive medicine or sperm handling procedures. The NMN is a naturally occurring intermediate in the nicotinamide adenine dinucleotide (NAD⁺) biosynthetic pathway and has attracted considerable attention because of its central role in cellular energy metabolism, mitochondrial function, and redox homeostasis (Yoshino et al., 2018). In preclinical reproductive studies, NMN has demonstrated beneficial effects in several animal models (Liu et al., 2025, Noh et al., 2025, Zhu et al., 2024). The NMN supplementation improved porcine sperm quality during storage by modulating the SIRT3-SOD2/ROS axis and oxidative phosphorylation (Zhang et al., 2025). Likewise, NMN at 60 μ M improved chilled ram sperm quality and reduced oxidative stress and apoptosis during short-term storage (Zhu et al., 2024). More recently, NMN was reported to protect human spermatozoa against microplastic-induced oxidative damage by preserving sperm motility, mitochondrial activity, membrane integrity, and early embryonic developmental potential under experimental conditions (Ren et al., 2026). These findings suggest that NMN may be biologically compatible with sperm cells and potentially protective under oxidative stress conditions.

However, these protective effects were observed either under induced stress conditions or in sperm of abnormal animals. Evidence regarding the safety of direct NMN exposure on normal human spermatozoa remains absent. With the increasing use of NMN as an anti-aging and fertility-supporting

supplement, evaluating its potential reprotoxicity is critical. The HSA is a well-established in vitro screening tool for assessing compound compatibility with human spermatozoa by monitoring progressive motility under standardized conditions (Nijs et al., 2009). Previous studies have reported considerable variation in assay duration, ranging from 4 hours for sperm stress testing to 24–48 hours in conventional sperm bioassays (Hossain et al., 2010). Accordingly, the HSA provides a practical and biologically relevant approach for evaluation of compounds intended for reproductive or antioxidant applications. The present study was designed as a preliminary pilot HSA to evaluate the compatibility and potential concentration-dependent reprotoxic effects of NMN on human spermatozoa. In this study, sperm progressive motility was assessed following exposure to 50, 100, or 150 μ M NMN concentrations selected based on prior sperm-related NMN studies.

Materials and Methods

Seven normozoospermic semen samples labeled A–G were obtained from donors with normal baseline progressive motility. Following liquefaction and routine semen evaluation, each sample was divided into 4 groups. The control as untreated and three NMN-treated groups. The effects of NMN was tested at 50 μ M, 100 μ M, or 150 μ M. The doses was selected according to the recent studies. The doses 50 and 100 μ M were selected because they fall within or near to concentrations used in prior sperm-related NMN experiments, while dose of 150 μ M was included to identify any threshold at which motility impairment might emerge. Moreover, the samples were incubated under standard laboratory conditions and assessed at baseline, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, and 24 hours. The progressive motility was evaluated according to WHO 2021 criteria at each time point.

Results

In this pilot Human HSA, the effects of NMN on progressive sperm motility were evaluated in 7 normozoospermic semen samples treated with NMN at 50 μ M, 100 μ M, and 150 μ M, then, progressive motility was assessed in different time points.

Exposure to 50 μ M and 100 μ M NMN did not impair progressive motility in any of the seven samples throughout the 24-hour incubation. Motility in these groups remained comparable to, or occasionally exceeded, that of untreated controls (Fig. 1). Regarding the primary aim of this study was to screen the sperm reprotoxicity, the observed beneficial effects on motility were not the primary focus of this pilot safety study; therefore, any improvements observed remain to evaluate in our future studies. In contrast, 150 μ M NMN induced an obvious reduction in progressive motility relative to controls (Fig. 2). Normalized motility indices (baseline = 100%) confirmed this pattern, demonstrating the preservation at lower concentrations and a marked decline at the highest concentration. These findings were consistent across all individual sample profiles.

Discussion

This study provides a preliminary HSA-based evaluation of NMN safety in human spermatozoa. The main finding is that NMN at 50 μM and 100 μM did not induce detectable impairment in progressive motility up to 24 hours of in vitro incubation. In contrast, 150 μM indicated reduced progressive motility relative to the untreated control, suggesting a concentration-dependent adverse effect on sperm function.

This concentration-dependent pattern is biologically plausible in light of the inherent susceptibility of human spermatozoa to xenobiotic and oxidative perturbations. Sperm cells possess a plasma membrane enriched in polyunsaturated fatty acids and relatively limited cytoplasmic antioxidant reserves, making motility one of the earliest and most sensitive functional readouts of toxic exposure. In this context, the reduction in progressive motility observed at 150 μM suggests that NMN may remain well tolerated within a lower exposure window, whereas higher concentrations may exceed the threshold at which sperm function begins to deteriorate.

Beneficial effects of NMN have been reported in several animal sperm models. In boar semen, NMN improved sperm quality during liquid storage by preserving mitochondrial function (Liu et al., 2025). In another porcine study, NMN enhanced sperm quality through activation of the SIRT3–SOD2/ROS pathway and promotion of oxidative phosphorylation (Zhang et al., 2024). Likewise, NMN improved the quality of chilled ram sperm by reducing oxidative stress and apoptosis during short-term storage (Zhu et al., 2024). More recently, NMN protected human spermatozoa against microplastic-induced oxidative injury by preserving sperm motility, mitochondrial activity, membrane integrity, and early embryonic developmental potential (Ren et al., 2026). Overall, the available evidence suggests that NMN may preserve sperm function under conditions associated with oxidative stress and sperm handling. These findings also suggest that the biological effects of NMN depend on both exposure concentration and the physiological conditions under which spermatozoa are evaluated. Whereas previous studies evaluated sperm under oxidative or environmental stress (Ren et al., 2026), the present study assessed the direct effects of NMN on normozoospermic human spermatozoa under standardized in vitro conditions using the HSA, a well-established screening assay for the preliminary evaluation of reproductive toxicity. By monitoring progressive motility under controlled conditions, the HSA provides a sensitive and practical assessment of sperm functional integrity (Nijs et al., 2009). Notably, the assay identified a concentration-related decline in progressive motility while demonstrating that lower NMN concentrations were well tolerated throughout the incubation period.

The concentrations of 50, 100, and 150 μM were selected to approximate biologically relevant exposures and to remain aligned with the range used in previous sperm-focused NMN studies, whereas 150 μM was included to explore whether a higher dose would reveal a measurable decrement in sperm performance. The finding that only the highest concentration impaired motility supports the existence of a potential exposure threshold, above which the net biological response may shift from neutral or favorable to unfavorable. Such concentration-dependent behavior is consistent with fundamental principles of reproductive toxicology, in which the biological response to a compound may vary according to exposure level and the physiological characteristics of the target cell. Although the present

findings require confirmation in larger cohorts and with additional functional and mechanistic endpoints, they provide an initial concentration range for future investigations aimed at defining the reproductive safety profile of NMN in human spermatozoa

Conclusion

This preliminary HSA found NMN at 50 μ M and 100 μ M does not produce detectable damage to the sperm progressive motility. However, the concentration of 150 μ M was associated with reduced progressive motility, suggesting a possible dose-dependent toxic effect on sperm function. These results support the NMN at lower concentrations is a safe chemical for use in sperm preparation and handling protocols.

Declarations

Authors contributions T.A: performed the experiments. S.A.S: performed the experiments. S.V: revising the final version of the manuscript. M.E.R. revising the final version of the manuscript and M.I: supervisor, validation of data, and revising the final version of the manuscript. All authors reviewed the manuscript. The authors declare that all data were generated in-house and that no paper mill was used

Data availability All data were produced by the authors during the course of this research, and no paper mill or third-party manuscript generation service was involved in the preparation of this manuscript.

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Declarations The authors report no conflicts of interest related to this study or its publication.

Ethics Approval This study was approved by the Institutional Ethics Committee of [Yazd University of Medical Sciences, Yazd, Iran according to the SSU ethics committee guidelines] (Approval No. IR.SSU.REC.1404.147). All procedures involving human participants were conducted in accordance with the ethical standards of the institutional research committee. **Participation** Informed consent forms for participation were signed by all couples.

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Figures

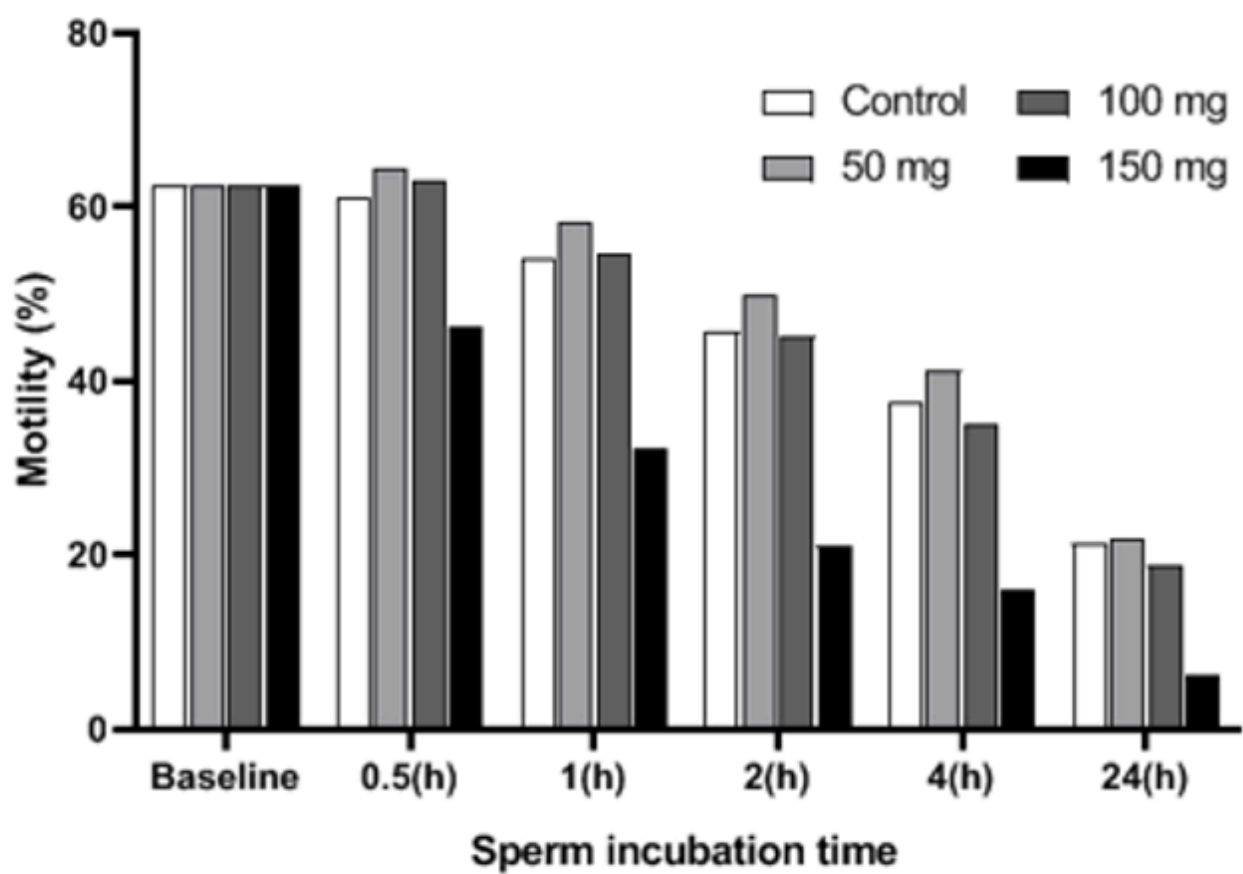


Figure 1

Progressive motility (%) of human spermatozoa following exposure to NMN (0, 50, 100, or 150 μ M) throughout the 24 hours in different samples.

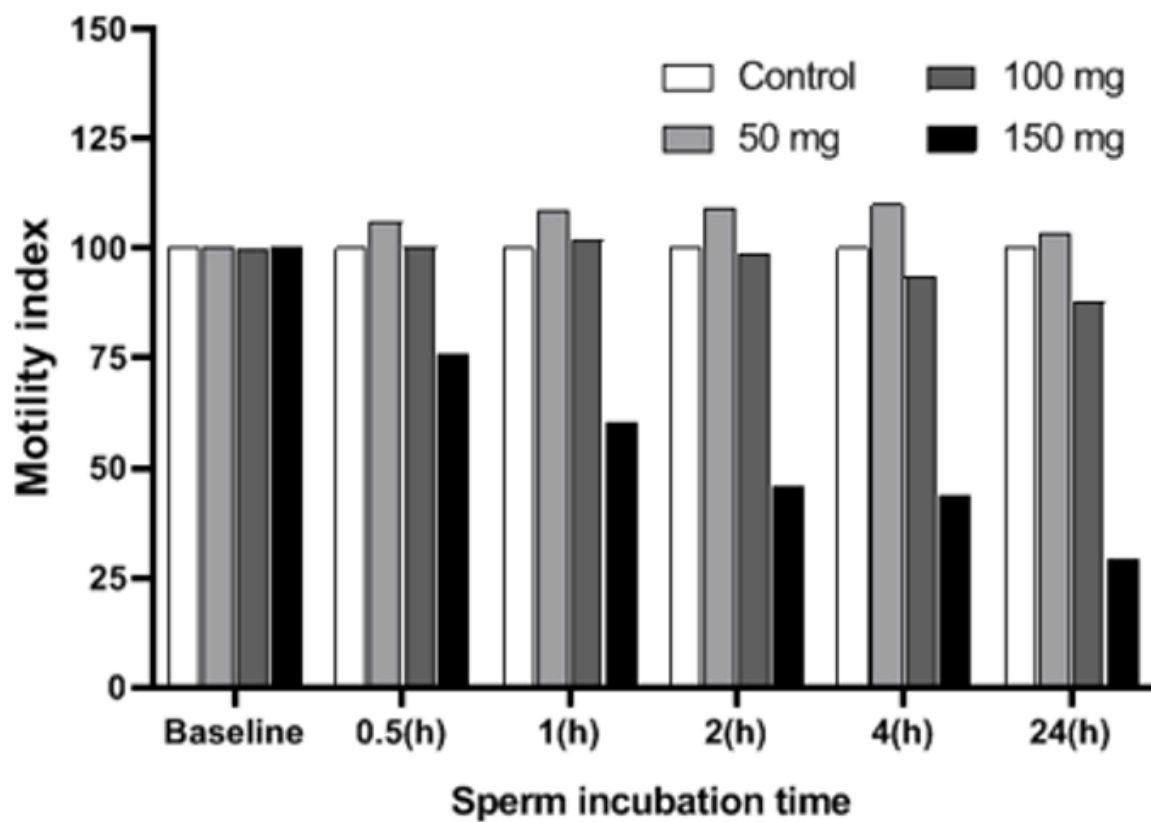


Figure 2

motility index (baseline = 100%) for control and NMN-treated groups across all samples (A–G). Data indicate concentration-dependent effects, with preservation of progressive motility at 50 and 100 μ M versus reduction thereof at 150 μ M.