DOI: 10.18502/jfrh.v19i4.21078

Original Article

Evaluating the Impact of Hyaluronic Acid on Sperm Quality Before and After Cryopreservation in A Moroccan Cohort: A Pilot Study

Mehdi EL Mokaddem; M.Sc.[1], Zeineb Zian; Ph.D.[1], Amr Kchikich; Ph.D.[2], Noureddine Louanjli; Ph.D.[3], Naima Ghailani Nourouti; Ph.D.[1], Amina Barakat; Ph.D.[1], Moudou M. Mbaye; Ph.D.[3], Mohcine Bennani Mechita; Ph.D.[1]

1 Intelligent Automation and BioMed Genomics Laboratory, Faculty of Sciences and Techniques of Tangier, Abdelmalek Essaadi University, Tetouan, Morocco
2 Laboratory of Biotechnological Valorization of Microorganisms, Genomics, and Bioinformatics, Faculty of Sciences and Techniques, University Abdelmalek Essaadi, Tetouan, Morocco
3 Laboratory of Medical Analysis, Andrology Department, LABOMAC, Casablanca, Morocco

Received October 2025; Revised and accepted December 2025

Abstract

Objective: This study aimed to evaluate the effects of hyaluronic acid (HA) supplementation on motility, vitality, and DNA integrity in Moroccan fresh and cryopreserved sperm samples.

Materials and methods: A cohort of 60 male participants was divided into three groups, including normozoospermic, asthenozoospermic, and cryopreserved normozoospermic samples. Advanced microscopy and computer-assisted semen analysis (CASA) were used to analyze the sperm parameters before and after HA supplementation. DNA fragmentation was analyzed using TUNEL assay, while chromatin condensation was evaluated by aniline blue staining. Statistical analysis was performed using ANOVA and Duncan's test.

Results: HA supplementation improved sperm motility and vitality ($p < 0.05$) in the normozoospermic group without altering DNA fragmentation or chromatin condensation. In the asthenozoospermic group, motility and vitality were significantly improved ($p < 0.05$). In the cryopreserved normozoospermic group, HA improved motility and vitality after thawing ($p < 0.05$).

Conclusion: HA is a promising adjuvant for enhancing the quality of both fresh and cryopreserved sperm for ART without compromising DNA integrity. Studies need to be extended for further exploration of the mechanism of action and optimization of clinical protocols toward its use in routine practice, particularly for diverse populations and in the more difficult cases of infertility.

Keywords: Cryopreservation; Fertility; Hyaluronic Acid; Male infertility; Morocco

Introduction

Male infertility is a major global health issue and is responsible for almost half of the cases of couple infertility (1). Over the last few decades, a stable increase in the rates of male infertility was reported. A global data analysis between 1990 and 2019 revealed a marked increase in the age-standardized prevalence rates, particularly in high-middle and

Correspondence:
Mehdi EL Mokaddem
Email: elmehdi.elmokaddem@etu.uae.ac.ma

middle socio-demographic indices (2). Male infertility also has far-reaching implications on the emotional and psychological health of the affected men and their partners, where defects of mobility or Asthenozoospermia usually cause a fertility issue. Currently, assisted reproduction techniques (ART), notably intra-cytoplasmic sperm injection (ICSI), represent the most possible line of treatment (3). Sperm quality in motility and DNA integrity are key factors for the male reproductive potential. However, such parameters are often compromised in azoospermic males, presenting serious challenges for clinical management. While various diagnosing and action techniques have made good development in ART administration, male infertility remains complex in its management. Sperm DNA fragmentation and chromatin condensation further reduce functionality and fertilization, exacerbating reproductive difficulties (4).

DNA fragmentation is strongly linked to motility and fertilization capability failures (5). High fragmentation rates are correlated with reduced success with ART, such as IVF and ICSI, which also influence the outcomes of pregnancy and live births (6,7). Similarly, poor chromatin condensation can damage the structural stability of sperm DNA, making it more susceptible to oxidative damage. This mechanism is a significant challenge during cryopreservation because it is an oxidative stress process that amplifies DNA damage (8).

Fertility preservation and ART are precious components of cryopreservation, with the deleterious complications of oxidative damage, cellular injury from freezing and thawing cycles, and generally poor semen quality, reduced motility, and vitality. Thus, interventions are urgently needed to reduce these effects and improve post-thawing viability. Hyaluronic acid (HA), a naturally occurring glycosaminoglycan found in the reproductive tract (9), has emerged as a favorable candidate for addressing these challenges. With its unique β-d-glucuronic acid and N-acetyl β-d-glucosamine structure, HA promotes cellular adhesion and growth regulation and modulates oxidative stress by many bioactive effects (10). Published studies documented the beneficial effects of HA on sperm quality. HA was shown to maintain sperm motility and membrane integrity upon thawing in both human and animal models, including equine and boar sperm (11-13). HA treatment can restore sperm motility and vitality under oxidative stress, a prevalent condition when dealing with sperm from natural and cryopreserved environments (14, 15). The role of HA in promoting capacitation and protecting sperm from oxidative damage highlights its therapeutic potential in ART (5).

Despite the growing body of evidence supporting the benefits of HA in improving sperm quality, no studies to date have investigated its effects within a Moroccan population. This study evaluates the impact of HA supplementation on key sperm parameters, including motility, vitality, DNA fragmentation, and chromatin condensation, across three patient groups. Group 1 (normozoospermic) served as a baseline, Group 2 (asthenozoospermic) focused on motility improvement, and Group 3 assessed HA's protective effects on cryopreserved sperm.

Materials and methods

This study was conducted in a private medical analysis and reproductive biology laboratory in accordance with the ethical principles of the Declaration of Helsinki and with local regulations for biomedical research. All procedures were performed on routine diagnostic samples, and no modification of patient management was undertaken for the purpose of this study. All data were fully anonymized before analysis, and written informed consent was obtained from all recruited individuals for the use of their anonymized data for research purposes. Samples were collected at the laboratory of medical analysis and Biology of Reproduction, “Labomac”, Casablanca, Morocco. Sixty male participants were included and divided into three subgroups. Subgroup 1 represented 20 normozoospermic patients (concentration $\geq 16 \times 10^6$ / ml, progressive motility $\geq$ 30%), and Subgroup 2 included 20 asthenozoospermic patients (concentration $\geq 16 \times 10^6$ / ml, progressive motility <30%), while Subgroup 3 represented 20 normozoospermic patients undergoing cryopreservation (-196 °C) (Figure 1).

For the liquefaction step, the samples were incubated at 37 °C under 5% CO_2 until analysis. Microscopic observations were performed according to standards and guidelines of the World Health Organization (WHO) (16).

For fresh samples (subgroups 1 and 2), sperm analysis was carried out one hour after semen production to determine the sperm count. Motility was assessed using a Makler counting chamber of 20 µm, while vitality was evaluated separately using appropriate staining methods. Sperm pre-treatment was performed by optimizing the density gradient technique.

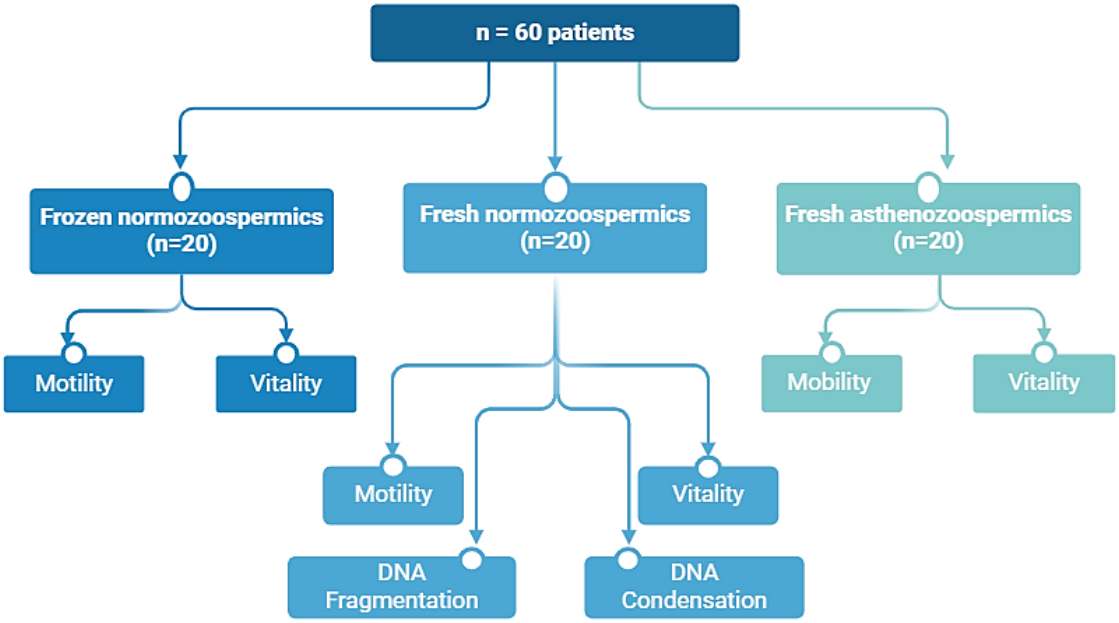

Figure 1: Flowchart of study population, created in BioRender.com

To prepare the sperm sample, 1 mL of 70% pure sperm solution was first added to a 5 ml falcon tube, followed by 1 mL of 45% pure sperm solution and 1 mL of the sperm sample. This ensures a clear separation of sperm fractions during process preparation. Thereafter, the tube was centrifuged at 500 rpm for 20 minutes. The remaining sperm fraction at the bottom of the tube for both samples was resuspended in 500µl of EARL (Physiological Solution Rich in Lipids), and the set was divided into three equal aliquots in 5 ml falcon tubes. The first tube containing the sperm fraction mixed with EARL was incubated at room temperature as a control (CTR), and the other two remaining tubes (HA1 and HA2) were incubated at room temperature with 0.875 mg/ml and 0.435 mg/ml of HA, respectively (Figure 2).

The effect of HA on human sperm motility was monitored at room temperature within various storage times (30, 60, 90, 120, 150, 180 minutes, 24, and 48 hours). The evaluation of HA's impact on sperm motility was conducted following the 2021 WHO recommendations (16). An aliquot of 20 µl of the mixture (sperm/HA1, sperm/HA2, or control) was placed on a Makler counting chamber of 20 µm. Immediately after preparing the mixture, the motility was assessed using Computer Assisted Semen Analysis (CASA) Hamilton-Thorne version 10 HTM IVOS Analyzer (Hamilton-Thorne Biosciences, Beverly, MA, USA).

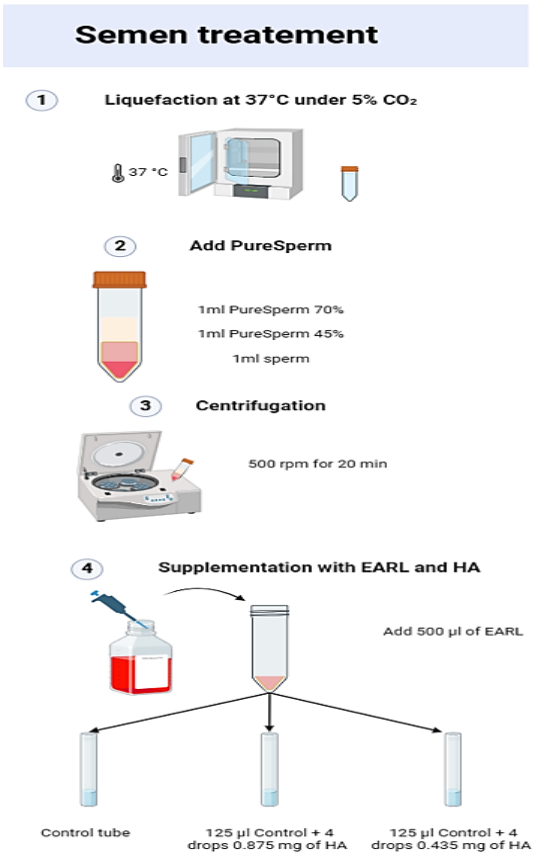

Figure 2: Fresh sperm treatment. Created in BioRender.com

The evaluation of sperm vitality was performed with eosin staining solution 2% (17). A drop of sperm was mixed with a drop of eosin solution 2% and spread between the coverslip and a slide. After the mixture was deposed, the preparation was observed under a light microscope. At least 100 spermatozoa per sample were analyzed under the microscope in replicates to calculate the frequency of viable sperm.

Sperm DNA integrity was assessed using the TUNEL assay commercial kit (Roche Diagnostics, Lewes, UK). 10 µL aliquot of each sample (sperm/HA-treated or control) was used to prepare a smear on a clean slide. The slides were fixed with 1/10 diluted formal solution with phosphate-buffered saline (PBS) for 30 minutes at room temperature and permeabilized using Triton X-100 in PBS to allow dye penetration. The TUNEL reaction mixture was prepared and applied according to the manufacturer's instructions, and samples were incubated following the recommended temperature and period. Fluorescence analysis was performed using a fluorescence microscope (Nikon Eclipse 80i). Results were expressed as the percentage of TUNEL-positive spermatozoa, indicating DNA fragmentation. For each sample, at least 100 spermatozoa were analyzed microscopically in replicates to calculate the frequency, thus ensuring statistical reliability and representativeness. DNA fragmentation analysis was excluded for subgroup 2 to avoid confounding factors, as the minimal changes in subgroup 1 samples suggested no clinically relevant risks in the broader context.

Sperm DNA condensation assessment was performed using aniline blue staining 2% (18). An aliquot of 10 µL from each sample (sperm/HA-treated and CTR) was used to prepare a smear on a clean slide. The smears were air-dried and then fixed with glutaraldehyde 3% in PBS for 30 minutes at room temperature. After fixation, the slides were stained with aniline blue aqueous solution 2% (pH 3.5) for 30 minutes. After staining, the slides were carefully washed with distilled water, and then air-dried. Stained spermatozoa were examined under a light microscope at 1000x magnification using oil immersion. Spermatozoa with dark blue nuclei were classified as having abnormal chromatin condensation, while those with pale or unstained nuclei were considered to have normal chromatin condensation. For each sample, at least 100 spermatozoa were analyzed under a microscope in replicates to calculate the percentage. It was expressed as the percentage of spermatozoa with abnormal chromatin condensation in each sample.

For cryopreserved samples (subgroup 3), sperm pre-treatment followed the density gradient optimization technique aforementioned described. The prepared sample was centrifuged, and the resulting fraction was collected for further cryopreservation. These fractions were then mixed with Spermfreeze solution and prepared for long-term storage in 250 µL cryovials, which were properly sealed. The cryovials were then placed in a controlled-rate freezer, where the temperature was gradually lowered from 25°C to -7°C for 10 minutes, followed by further cooling from -7°C to -30°C before being immersed in liquid nitrogen at -196°C for long-term storage (19, 20) (Figure 3).

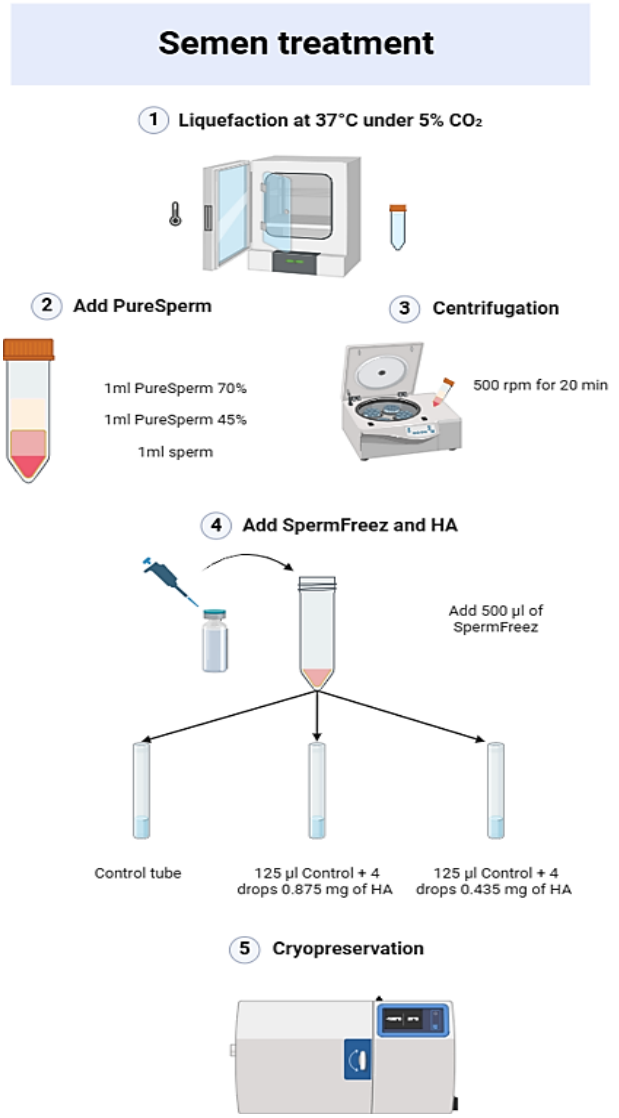

Figure 3: Sperm treatment for cryopreserved samples

For the thawing of cryopreserved samples, the cryovial is removed from the liquid nitrogen tank to gradually reach room temperature. Once the vial has reached ambient temperature, the cap is carefully removed, and the contents are transferred into a 5 mL hemolysis tube. Then, 3 to 4 drops of EARL are added to the tube, which is quietly shaken to mix the solution. Sperm motility was assessed using CASA, and eosin staining for vitality, following the same methods applied to fresh semen.

Statistical analysis: Percentage data were arcsine square root transformed (√p) for normalization. All data were analyzed using a general linear model (GLM) in SAS 9.4 software, accounting for the fixed effects of treatment (HA concentration) and storage duration. Mean comparisons were performed using the Duncan test. Pearson correlation coefficients were calculated to study relationships between variables. A p-value < 0.05 was considered statistically significant.

Results

The effect of HA supplementation on sperm motility was assessed in subgroups 1 and 2 (normozoospermic and asthenozoospermic samples, respectively). In subgroup 1, HA supplementation significantly enhanced sperm motility compared to the control group ($p < 0.05$). A significant improvement in motility was observed with HA supplementation starting from T150 for both concentrations (H1, 0.875 mg/ml and H2, 0.435 mg/ml). Unlike the lower concentration (H2, 0.435 mg/ml), a significant effect of storage time was observed for the higher concentration of HA (H1, 0.875 mg/ml). However, a significant improvement in motility was observed after T180, driven by the storage time.

In subgroup 2, the effect of HA was notable earlier, with a significant improvement in motility observed from T90 for both concentrations compared to the control group ($p < 0.05$). Additionally, a significant effect of storage time was observed starting from T120.

The effect of HA on sperm vitality was evaluated in subgroups 1 and 2. In both subgroups, HA supplementation significantly improved sperm vitality compared to the control group ($p < 0.05$). However, no significant effect of HA concentration (H1, 0.875 mg/ml, and H2, 0.435 mg/ml) was observed. On the other hand, a significant effect of storage time on sperm vitality was noted starting from T60 for both concentrations in both subgroups

The impact of HA supplementation on sperm DNA fragmentation was evaluated using the TUNEL assay in subgroup 1. No significant effect of concentration was observed for either H1 or H2. However, a significant effect of storage time was noted, with DNA fragmentation increasing starting from T60.HA treatment resulted in a statistically significant increase in DNA fragmentation *($p < 0.05$).* Spermatozoa with fragmented DNA (TUNEL-positive) exhibited bright green or yellow fluorescence, while spermatozoa with intact DNA (TUNEL-negative) showed pale yellow fluorescence (Figure 4).

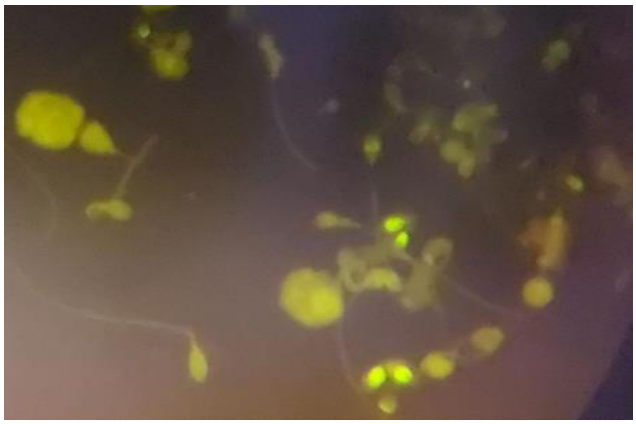

Figure 4: Assessment of sperm DNA fragmentation Normozoospermic subgroup using the TUNEL Assay

The results of the effect of HA on sperm DNA condensation in subgroup 1 showed that a statistically significant change was detected post-HA treatment *($p < 0.05$).* However, no significant effect of evident from T60 for the H1 concentration and T90 for the H2 concentration (Figure 5).

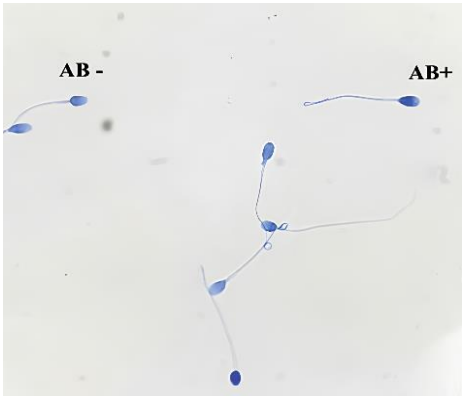

Figure 5: Assessment of sperm DNA condensation Normozoospermic subgroup using 2% Aniline blue staining

The impact of HA supplementation on the motility and vitality of frozen sperm was assessed in Subgroup 3. Following thawing and HA supplementation, sperm motility and vitality showed a significant improvement compared to the control group *(p* < 0.05*)*. Both concentrations (H1 and H2) had a significant effect on motility and vitality post-thawing, with H1 demonstrating a higher improvement index than H2.

Vitality showed a strong positive correlation with motility (r = 0.86; *p* < 0.05), indicating that sperm with higher vitality were more likely to have improved motility. Conversely, vitality was moderately and negatively correlated with DNA condensation (r = -0.58; *p* < 0.05) and DNA fragmentation (r = -0.68; *p* < 0.05). Motility also showed a moderate negative correlation with DNA condensation (r = -0.52; *p* < 0.05) and a strong negative correlation with DNA fragmentation (r = -0.71; *p* < 0.05).

DNA condensation was positively correlated with DNA fragmentation (r = 0.62; *p* < 0.05). The correlation coefficients between sperm quality parameters are presented in Table 1.

Discussion

HA has emerged as a key factor in improving sperm parameters for ART. By creating a physiological environment that mimics the oocyte-cumulus complex, HA improves sperm motility and vitality while preserving DNA integrity, including reduced fragmentation and improved chromatin condensation (21). On the other hand, human sperm cryopreservation plays a crucial role in fertility preservation strategies, especially for patients undergoing medical treatments that may affect their reproductive potential. This study, conducted on a Moroccan cohort, provides new insights into the effect of HA supplementation on sperm quality post-thawing.

Our results showed that HA concentrations of 0.435 mg/ml and 0.875 mg/ml are particularly effective in improving sperm parameters. Previous research has reported similar beneficial effects on the same parameters using concentrations of 0.750 mg/ml, 1 mg/ml, and 1.25 mg/ml (22). These concentrations select mature sperm with intact chromatin and acrosomes, which are essential key factors for successful fertilization (21, 23). Higher concentrations may impair motility due to increased viscosity, reflecting the need for adequate concentration adjustments (22).

In our work, HA1 and HA2 improved sperm motility compared to control groups (p < 0.05) in both normozoospermic and asthenozoospermic subgroups. This suggests that HA may enhance spermatozoa function by creating a physiologically relevant environment for their motility mechanisms and survival (24, 25). Jakab et al. indicated that sperm selected through HA binding showed reduced chromosomal aneuploidies (26), supporting the hypothesis that HA-targeted selection would enhance sperm quality at structural and genetic levels.

Similarly, HA supplementation increased sperm viability in both normozoospermic and asthenozoospermic subgroups (p < 0.05). Storage time and concentrations of HA influenced the notable effect on viability, showing the role of time in improving sperm functionality after HA treatment. This is consistent with the assumption of the membrane-stabilizing role of HA on sperm, by reducing oxidative stress (23). Other findings further emphasize the high specificity of HA in its interaction with sperm receptors, serving to exclude apoptotic or necrotic sperm (27).

HA1 and HA2 supplementation on the normozoospermic samples significantly increased DNA fragmentation (p < 0.05), modulated by the storage time and HA concentration. However, these levels remained below the pathological threshold value of 30% (28). Our results corroborate previous studies demonstrating that HA-bound sperm showed lower risks for DNA damage and chromosomal abnormalities (29). Similar results had been documented, in which modified DNA condensation was noted after HA supplementation (p<0.05), however, fell within normal physiological limits. Thus, our findings demonstrate that HA does not cause clinically significant DNA damage.

Table 1: Correlation coefficients between semen quality parameters (n = 20)

	Vitality	Motility	Chromatin condensation	DNA fragmentation
Vitality	1	0.86***	-0.58***	-0.68***
Motility		1	-0.52***	-0.71***
Chromatin condensation			1	0.62***
DNA fragmentation				1

The significant correlation coefficients analysis was set at ***p< 0.001

This implies that HA binding is strongly related to sperm maturation, including proper chromatin remodeling and protamine exchange, emphasizing its role in maintaining chromatin integrity (24).

Sperm DNA integrity, which incorporates fragmentation and chromatin condensation, is essential to sperm function and fertility. DNA fragmentation has been correlated with lower motility, probably due to oxidative stress-induced damage impairing flagellar movement and general sperm performance (5). Similarly, chromatin condensation is directly correlated to motility since insufficient chromatin condensation is tightly linked with abnormal motility owing to defective chromatin packaging, accompanied by structural instability resulting in compromised sperm function (5, 29). The lack of significant alteration in fragmentation and condensation reported in the current study shows the relevant impact of HA in maintaining DNA integrity while enhancing motility. This suggests that HA has created a physiological environment to preserve both structural and functional sperm parameters that enhance its probable applications for therapy in ART.

The effect of HA on post-thaw sperm motility and viability was remarkable. HA1 and HA2 supplementation significantly improved these same parameters compared to the control group ($p < 0.05$), with HA1 giving more pronounced improvements. These results support that HA may provide protection for sperm functionality during cryopreservation and mark a potential application within ART that could specifically attend to the characteristics of the population studied. Indeed, these results support previous reports (11) showing enhanced motility retention in cryopreserved human sperm treated with HA. Additionally, studies in other species provided acknowledgements of HA's effect in preserving post-thaw sperm quality, such as rams (30), bulls (31), and goats (32). Previous published works (12, 33) have also confirmed that HA protects stallions and boars from damage to membrane and motility. The physiological activity behind the protective role of HA includes forming a vitreous coating around sperm cell membranes during freezing, protecting against physical damage, and preserving acrosome integrity (34). This minimizes cryo-induced injury to the sperm and thus maintains their function, especially when thawed. HA is also documented to modulate capacitation processes and maintain membrane function (35). It must be noted that HA delivers critical effects on sperm viability and fertilizing potentials alike after thawing. Some studies show that HA fails to exert systematic improvements in post-thaw sperm quality in ram cryopreserved samples (30). These studies imply that HA must thus be grouped with such factors as the species, freezing procedures, and concentration, which determine its protective action (36). Furthermore, no improvement was reported in ram sperm treated with HA post-thaw (37). These findings underline the variability in HA's effects and the need for optimized protocols tailored to specific conditions.

The capabilities of HA concentrations (HA1 and HA2) to enhance sperm motility and viability in both fresh and cryopreserved samples strongly stress its significance in ART. By protecting membrane integrity, HA preserves post-thaw quality and fertility via the maintenance of optimal functionality, consistent with findings in human and animal studies. The variability in outcomes shows that improvements would be strengthened by elevating HA concentrations or changing the cryopreservation conditions, which could maximize its efficacy. More investigations are necessary to better elucidate the mechanisms offered by HA and understand its other clinical applications.

The present study has some limitations that should be acknowledged. First, the small sample size of our series could limit the generalizability of the results. Another limitation is that the HBA test, which measures the ability of spermatozoa to bind HA, is used as an indicator of spermatozoa maturation and physiological ability. Another key determinant of sperm quality, the measurement of reactive oxygen species (ROS) and total antioxidant capacity (TAC) would have provided clearer conclusions on HA's potential role in reducing or increasing oxidative stress. While our study showed preservation in both motility and viability, its impact on the fertilization potential was not assessed. Evaluating whether these preserved parameters translate into improved fertilization and embryo development outcomes could be crucial. All these considerations open up new possibilities for an overall assessment of men's reproductive health.

Conclusion

In the present study, we explored the potential of HA as a therapeutic agent to enhance major sperm parameters in a Moroccan series. Our results showed significantly higher preservation of sperm motility and vitality with HA1 and HA2 in both

normozoospermic and asthenozoospermic samples as compared to their respective control groups, as well as in cryopreserved sperm post-thawing. These findings support the multiple therapeutic advantages of HA in treating male infertility without compromising the integrity and quality of DNA. Future research should focus on larger cohort studies and developing advanced molecular investigations to further refine HA-based treatments and enhance their clinical efficacy.

Conflict of Interests

Authors declare no conflict of interests.

Acknowledgments

None.

References

1. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015;13(1):1–9.
2. Huang B, Wang Z, Kong Y, Jin M, Ma L. Global, regional and national burden of male infertility in 204 countries and territories between 1990 and 2019: an analysis of Global Burden of Disease Study. BMC Public Health. 2023;23(1).
3. Cavarocchi E, Whitfield M, Drouault M, Touré A, Ribeiro JC, Simon V. Human asthenozoospermia: Update on genetic causes, patient management, and clinical strategies. 2025:1–21.
4. Stavros S, Potiris A, Molopodi E, Mavrogianni D, Zikopoulos A, Louis K, et al. Sperm DNA fragmentation: Unraveling its imperative impact on male infertility based on recent evidence. Int J Mol Sci. 2024;25(18).
5. Agarwal A, Virk G, Ong C, du Plessis SS. Effect of oxidative stress on male reproduction. World J Mens Health. 2014;32(1):1.
6. Sharma R, Iovine C, Agarwal A, Henkel R. TUNEL assay—standardized method for testing sperm DNA fragmentation. Andrologia. 2021;53.
7. Agarwal A, Baskaran S, Parekh N, Cho CL, Henkel R, Vij S, et al. Male infertility. Lancet. 2021;397:319–33.
8. Hallam J, Burton P, Sanders K. Poor sperm chromatin condensation is associated with cryopreservation-induced DNA fragmentation and cell death in human spermatozoa. J Clin Med. 2024;13(14).
9. Fouladi-Nashta AA, Raheem KA, Marei WF, Ghafari F, Hartshorne GM. Regulation and roles of the hyaluronan system in mammalian reproduction. Reproduction. 2017;153(2):R43–58.
10. Simulescu V, Kalina M, Mondek J, Pekař M. Long-term degradation study of hyaluronic acid in aqueous solutions. Carbohydr Polym. 2016;137:664–8.
11. Sbracia M, Grasso J, Sayme N, Stronk J, Huszar G. Hyaluronic acid substantially increases retention of motility in cryopreserved human spermatozoa. Hum Reprod. 1997;12(9):1949–54.
12. Baumber J, Ball BA, Gravance CG, Medina V, Davies-Morel MCG. Effect of reactive oxygen species on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and lipid peroxidation. J Androl. 2000;21(6):895–902.
13. Qian L, Yu S, Zhou Y. Protective effect of hyaluronic acid on cryopreserved boar sperm. Int J Biol Macromol. 2016;87:287–9.
14. Bansal AK. Antioxidants and other potent strategies to reduce oxidative stress in semen. In: Free Radicals in Human Health and Disease. Springer India; 2015. p. 381–95.
15. Marchlewska K, Erkiert-Kusiak M, Walczak-Jędrzejowska R, Słowikowska-Hilczer J. Sperm migration and hyaluronic acid binding: implications for male fertility evaluation. Int J Mol Sci. 2024;25(18).
16. Chung E, Arafa M, Boitrelle F, Kandil H, Henkel R, Saleh R, et al. The new 6th edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen. Asian J Androl. 2022;24:123–4.
17. Björndahl L, Söderlund I, Kvist U. Evaluation of one-step eosin-nigrosin staining technique for human sperm vitality assessment. Hum Reprod. 2003;18(4):813–6.
18. Sharma R, Biedenharn KR, Fedor JM, Agarwal A. Lifestyle factors and reproductive health: taking control of your fertility. Reprod Biol Endocrinol. 2013;11.
19. Tao Y, Sanger E, Saewu A, Leveille MC. Human sperm vitrification: state of the art. Reprod Biol Endocrinol. 2020;18.
20. Huang C, Tang YL, Hu JL, Zhou WJ, Huang ZH, Luo XF, et al. Update on techniques for cryopreservation of human spermatozoa. Asian J Androl. 2022;24:563–9.
21. Huszar G, Jakab A, Sakkas D, Ozenci CC, Cayli S, Delpiano E, et al. Fertility testing and ICSI sperm selection by hyaluronic acid binding: clinical and genetic aspects. Reprod Biomed Online. 2007;14:650–63.
22. Roushandeh AM, Sohrabvand F, Amanpour S, Pasbakhsh P, Sobhani A, Nikzad H, et al. Effects of different doses of hyaloronan on human sperm motility, vitality and morphology. Acta Med Iran. 2009;47(5):369–72.

23. Parmegiani L, Cognigni GE, Bernardi S, Troilo E, Ciampaglia W, Filicori M. "Physiologic ICSI": hyaluronic acid favors selection of spermatozoa without DNA fragmentation and with normal nucleus. Fertil Steril. 2010;93(2):598–604.
24. Huszar G, Ozenci CC, Cayli S, Zavaczki Z, Hansch E, Vigue L. Hyaluronic acid binding by human sperm indicates cellular maturity, viability, and unreacted acrosomal status. Fertil Steril. 2003;79(Suppl 3):1616–24.
25. Saylan A, Abant B, Duman S. Efficacy of hyaluronic acid in the selection of human spermatozoa with intact DNA by the swim-up method. Cell J. 2016.
26. Jakab A, Sakkas D, Delpiano E, Cayli S, Kovanci E, Ward D, et al. Novel selection method for sperm with normal chromosomal aneuploidies during ICSI. Fertil Steril. 2005;84(6):1665–73.
27. Beck-Fruchter R, Shalev E, Weiss A. Clinical benefit of sperm hyaluronic acid binding in ICSI cycles: systematic review and meta-analysis. Reprod Biomed Online. 2016;32(3):286–98.
28. Venkatesh S, Singh A, Shamsi MB, Thilagavathi J, Kumar R, Mitra DK, et al. Clinical significance of sperm DNA damage threshold value in male infertility. Reprod Sci. 2011;18(10):1005–13.
29. Vozdova M, Oracova E, Kasikova K, Prinosilova P, Rybar R, Horinova V, et al. Balanced chromosomal translocations in men: relations among semen parameters, chromatin integrity, sperm meiotic segregation and aneuploidy. J Assist Reprod Genet. 2013;30(3):391–405.
30. Bucak MN, Ateşşahin A, Varişli Ö, Yüce A, Tekin N, Akçay A. Influence of trehalose, taurine, cysteamine and hyaluronan on ram semen after freeze-thawing. Theriogenology. 2007;67(5):1060–7.
31. Eidan SM. Effect of adding vitamin C with catalase or reduced glutathione to Tris extender on post-thaw semen of Holstein bulls. Anim Reprod Sci. 2016;167:1–7.
32. Salmani H, Nabi MM, Vaseghi-Dodaran H, Rahman MB, Mohammadi-Sangcheshmeh A, Shakeri M, et al. Effect of glutathione in soybean lecithin-based extender on goat semen quality after freeze-thawing. Small Rumin Res. 2013;112:123–7.
33. Peña FJ, Johannisson A, Wallgren M, Rodriguez-Martinez H. Antioxidant supplementation improves boar sperm motility and mitochondrial potential after cryopreservation. Anim Reprod Sci. 2003;78(1–2):85–98.
34. Singh A, Li P, Beachley V, McDonnell P, Elisseeff JH. Hyaluronic acid-binding contact lens with enhanced water retention. Contact Lens Anterior Eye. 2015;38(2):79–84.
35. Suzuki K, Asano A, Eriksson B, Niwa K, Nagai T, Rodriguez-Martinez H. Capacitation status and in vitro fertility of boar spermatozoa: effects of seminal plasma, COC-conditioned medium and hyaluronan. Int J Androl. 2002;25(2):84–93.
36. Erenpreiss J, Spano M, Erenpreisa J, Bungum M, Giwercman A. Sperm chromatin structure and male fertility: biological and clinical aspects. Asian J Androl. 2006;8:11–29.
37. Peris SI, Morrier A, Dufour M, Bailey JL. Cryopreservation of ram semen facilitates sperm DNA damage. J Androl. 2004;25(2):224–33.

Citation: EL Mokaddem M; Zian Z, Kchikich A, Louanjli N, Nourouti NG, Barakat A, et al. **Evaluating the Impact of Hyaluronic Acid on Sperm Quality Before and After Cryopreservation in A Moroccan Cohort: A Pilot Study.** J Family Reprod Health 2025; 19(4): 301-9.