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ORIGINAL PAPER

Body mass index and sperm morphology as independent predictors of increased sperm DNA fragmentation after cryopreservation in men with severe oligoasthenoteratozoospermia

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Summary *Background: Semen cryopreservation is vital for fertility preservation in men with oligoasthenoteratozoospermia (OAT). While cryopreservation increases sperm DNA fragmentation (SDF), patient-specific risk factors in severe OAT remain poorly defined. We aimed to quantify the change in DNA fragmentation index (DFI) and identify clinical predictors of susceptibility.*

Methods: This retrospective cohort study enrolled 340 men with severe OAT (sperm count < 5 million/mL, total motility < 42%, progressive motility < 30%, morphology < 4%). DFI was assessed in fresh and post-thaw samples using the TUNEL assay. Correlations and multivariate regression analyses evaluated relationships between DFI change and age, body mass index (BMI), sperm parameters, and smoking status.

Results: The mean DFI increased significantly from 22.0% (IQR 15.0-29.0%) in fresh semen to 36.0% (IQR 23.0-54.0%) post-cryopreservation ($p < 0.001$). The median absolute increase (ΔDFI) was 9.5% (IQR 6.0-25.0%). Univariate analysis identified significant correlations between the ΔDFI and higher BMI ($\rho = 0.544$, $p < 0.001$), lower sperm count ($\rho = -0.638$, $p < 0.001$), and poorer sperm morphology ($\rho = -0.669$, $p < 0.001$). In the final multivariate linear regression model, higher BMI and poorer sperm morphology remained the only significant independent predictors of a greater increase in DFI after cryopreservation (both $p < 0.001$). This model explained 93.7% of the variance in the ΔDFI (Adjusted $R^2 = 0.937$, $p < 0.001$). Age and sperm count were not independent predictors in the final model. Smoking status and varicocele grade were not significantly associated with the ΔDFI.

Conclusions: Cryopreservation significantly exacerbates sperm DNA damage in severe OAT, but this effect is not uniform. Higher BMI and poorer sperm morphology are independent risk factors for greater damage, identifying a vulnerable patient subgroup. These men may benefit from personalized counseling and optimized cryopreservation protocols to mitigate DNA damage and improve future assisted reproductive technology outcomes.

KEY WORDS: *Infertility; Male; Sperm; DNA fragmentation; Cryopreservation; Assay; Oligoasthenoteratozoospermia; Body Mass Index; Fertility preservation.*

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INTRODUCTION

Male factor infertility is a primary concern in approximately half of all infertile couples (1). The integrity of sperm DNA has emerged as a critical parameter beyond standard semen analysis, with elevated *sperm DNA fragmentation* (SDF) linked to reduced fertilization rates, impaired embryo development, and lower pregnancy rates following *assisted reproductive technologies* (ART) (2, 3).

For men facing gonadotoxic treatments, such as chemotherapy, or those with progressive testicular dysfunction, semen cryopreservation is an indispensable tool for fertility preservation (4, 5). This is particularly relevant for men with *oligoasthenoteratozoospermia* (OAT), for whom the availability of viable sperm is already limited. Advances in ART, especially *intracytoplasmic sperm injection* (ICSI), have made cryopreservation a feasible option even in cases of severe male factor infertility (6).

Despite its utility, the cryopreservation process itself induces cellular stress through osmotic shifts, ice crystal formation, and oxidative damage, leading to a well-documented decline in sperm motility, viability, and DNA integrity (7, 8). The TUNEL assay, which detects DNA strand breaks, has been widely used to quantify this damage (9).

While the detrimental effect of freezing on sperm DNA is established, a critical gap remains in understanding which patients are most susceptible. This is especially pertinent for men with severe OAT, whose sperm may have compromised baseline integrity. Therefore, this study aimed not only to assess the increase in *DNA fragmentation index* (DFI) post-cryopreservation in a severe OAT cohort but,

more importantly, to identify specific clinical and seminal predictors – such as age, BMI, and baseline semen parameters – that modulate the extent of this damage.

Methods

This retrospective cohort study was conducted at *Alexandria Main University Hospital* between March 2022 and August 2024. The study analyzed data from 340 men diagnosed with severe *oligoasthenoteratozoospermia* (OAT). Severe OAT was defined as: sperm concentration < 5 million/mL, total motility < 42%, progressive motility < 30%, and normal sperm morphology < 4%, according to the *World Health Organization* (WHO) laboratory manual for the examination and processing of human semen (6th edition, 2021).

According to the policies of Alexandria University and applicable national regulations, this research involved the analysis of existing, de-identified clinical data and biospecimens originally collected for diagnostic purposes. Therefore, it did not constitute human subjects research requiring formal *Institutional Review Board* (IRB) approval. Patient consent for the use of de-identified data in research was obtained per standard institutional protocol at the time of sample collection.

Exclusion criteria included: history of anabolic steroid use within the past year, current systemic corticosteroid or immunosuppressive therapy, confirmed genetic causes of infertility (e.g., karyotype abnormalities, Y-microdeletions), or acute febrile illness within the past 90 days.

For each participant, the following data were extracted from medical records: self-reported smoking status (quantified as pack-years), comorbidities (including diabetes, clinically graded varicocele, and hypogonadism), and current medications. Height and weight measurements were used to calculate *Body Mass Index* (BMI, kg/m²).

Semen analysis and cryopreservation protocol

A single semen sample per patient was obtained by masturbation after 2-7 days of sexual abstinence. After liquefaction at 37°C for 15-30 minutes, standard semen analysis was performed within 1 hour of ejaculation according to WHO 2021 guidelines by experienced laboratory personnel.

Cryopreservation was performed using a commercial sperm cryoprotectant (*SAGE, CooperSurgical, USA*). The cryoprotectant was added dropwise to achieve a 1:1 (v/v) dilution, followed by equilibration for 10 minutes at room temperature. Samples were frozen using a vapor-phase method: cooled at -20°C for 8 minutes, suspended in liquid nitrogen vapor for 2 hours, and finally stored in liquid nitrogen (-196°C).

For post-thaw analysis, all samples were cryopreserved for a period of 30 days. Samples were thawed in a 37°C water bath for 5 minutes, washed twice by centrifugation (300 × g, 10 minutes) using *Human Tubal Fluid* (HTF) supplemented with 5% *human serum albumin* (HSA), and resuspended for assessment.

DNA fragmentation was evaluated in fresh and post-thaw samples using the TUNEL assay (*In Situ Cell Death Detection Kit, Fluorescein, Roche Diagnostics, Germany*) according to the manufacturer's instructions. A minimum of 200 spermatozoa per sample were scored under fluorescence microscopy by a single blinded observer. The *DNA Fragmentation Index* (DFI) was calculated as the percentage of TUNEL-positive sperm.

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows, Version 28.0 (*IBM Corp., Armonk, NY, USA*). The Shapiro-Wilk test assessed normality. Continuous variables are presented as mean ± *standard deviation* (SD) or median and *interquartile range* (IQR), as appropriate. Categorical variables are presented as frequencies and percentages.

The primary outcome was the change in DFI (ΔDFI = post-thaw DFI – fresh DFI). The Wilcoxon signed-rank test compared paired DFI values. Spearman's rank correlation (ρ) assessed univariate relationships between ΔDFI and continuous variables. The Mann-Whitney U and Kruskal-Wallis H tests were used for group comparisons. A stepwise multiple linear regression model was constructed to identify independent predictors of ΔDFI, including variables with univariate $p < 0.10$. Model assumptions were verified, and multicollinearity was assessed using the *variance inflation factor* (VIF). The final model's explanatory power is reported as adjusted R^2. A two-tailed $p < 0.05$ was considered statistically significant.

Results

This study analyzed 340 men to assess the impact of cryopreservation on sperm DNA integrity and identify predictive factors for cryopreservation -induced damage.

The mean age of participants was 37.2 ± 8.9 years, and the mean BMI was 27.7 ± 4.8 kg/m², with 73.2% classified as

Table 1.
Baseline characteristics of the study Cohort (n = 340).

Characteristic	Value
Age (years)	
Mean ± SD	37.23 ± 8.89
Median (IQR)	37.5 (29.0-46.0)
Range	24.0-54.0
BMI (kg/m²)	
Mean ± SD	
Category: n (%)	27.70 ± 4.75
Normal	91 (26.8%)
Overweight	136 (40.0%)
Obese	113 (33.2%)
Smoking Status: n (%)	
Non-smoker	113 (33.2%)
Smoker	227(66.8%)
Clinical Varicocele Grade: n (%)	
Grade I	79 (23.2%)
Grade II	136 (40.0%)
Grade III	125 (36.8%)
Semen parameters	
Count (million/ejaculate): Mean ± SD	3.34 ± 1.08
Morphology (% normal): Median (IQR)	2.0 (2.0-2.0)
Motility (progressive motility, %): Mean ± SD	25.43 ± 4.59

IQR: interquartile Range; SD: Standard Deviation.

Table 2.
Sperm DNA Fragmentation Index (DFI) before and after cryopreservation.

Parameter	Fresh Sperm	Post-Cryopreservation	Difference (post-Fresh)	P-value
DFI, Median (IQR)	22.0 (15.0-29.0)	36.0 (23.0-54.0)	9.50 (6.0-25.0)	< 0.001
DFI, Mean ± SD	22.83 ± 8.40	39.64 ± 19.37	16.81 ±16.59	
DFI Range	12.0-40.0	16.0-100.0	2.0-86.0	

** Comparison by Wilcoxon Singed-Rank Test.*

overweight or obese. The majority (66.8%) were smokers. Baseline semen analysis revealed a mean sperm count of 3.34 ± 1.08 million per ejaculate and a median normal sperm morphology of 2.0% (IQR 2.0-2.0%) (Table 1).

Cryopreservation resulted in a significant increase in the sperm *DNA fragmentation index* (DFI). The median DFI rose from 22.0% (IQR 15.0-29.0%) in fresh samples to 36.0% (IQR 23.0-54.0%) post-cryopreservation ($p < 0.001$). The median absolute increase (ΔDFI) was 9.5 percentage points (IQR 6.0-25.0) (Table 2).

Univariate analysis identified significant correlations between the ΔDFI and higher BMI ($\rho = 0.544$, $p < 0.001$), lower sperm count ($\rho = -0.638$, $p < 0.001$), and poorer sperm morphology ($\rho = -0.669$, $p < 0.001$).

In the final multivariate linear regression model, higher BMI and poorer sperm morphology remained the only significant independent predictors of a greater increase in DFI after cryopreservation (both $p < 0.001$). This model explained 93.7% of the variance in the ΔDFI (Adjusted $R^2 = 0.937$, $p < 0.001$) (Table 3). Age and sperm count were not independent predictors in the final model. Smoking status and varicocele grade were not significantly associated with the ΔDFI.

Table 3.
Factors associated with the increase in DNA Fragmentation Index after cryopreservation.

A: Univariate Correlation (Spearman's ρ)		
Variable	**Correlation with ΔDFI (ρ)**	**p-value**
BMI (kg/m^2)	0.544	< 0.001
Sperm Count (million/ejaculate)	-0.638	< 0.001
Age (years)	0.381	< 0.001
Morphology (% normal)	-0.669*	< 0.001

B: Final Multiple Linear Regression Model for ΔDFI			
Independent Predictor	**Unstandardized coefficient (B)**	**Standardized coefficient (Beta)**	**p-value**
BMI	0.128	1.455	< 0.001
Sperm Morphology	-0.649	-0.557	< 0.001

Model Summary: Adjusted $R^2 = 93.7\%$, $F = 207.27$, $p < 0.001$.
ΔDFI: Change in DNA Fragmentation Index.
** Based on non-parametric group comparison (Kruskal-Wallis) shown in original data, confirming a strong negative relationship.*

Discussion

This study confirms the significant detrimental impact of cryopreservation on sperm DNA integrity in a large, well-defined cohort of 340 men with severe OAT – a patient population for whom fertility preservation is both crucial and challenging. The key novel finding is the identification of specific, modifiable patient factors that independently predict the degree of this iatrogenic damage.

The observed median increase in DFI from 22.0% to 36.0% ($p < 0.001$) aligns with the established pathophysiology wherein the freeze-thaw process induces oxidative stress and chromatin destabilization (7, 10). Our data, derived from a substantial cohort, robustly reinforce that this vulnerability is pronounced in men with pre-existing compromise in seminal parameters.

The primary contribution of this study is the development of a strong predictive model. We found that higher BMI and poorer sperm morphology are independent risk factors for a greater absolute increase in DFI (ΔDFI) post-cryopreservation, together explaining 93.7% of the variance in the outcome.

Our finding that elevated BMI is a strong independent predictor of increased DNA damage after cryopreservation adds a crucial dynamic perspective to the existing body of evidence. Recent studies have established a clear association between higher BMI and poorer baseline semen parameters, including chromatin quality (11, 14). Furthermore, research indicates this relationship may be mediated by oxidative stress and altered seminal antioxidant capacity (2). However, a notable study by Chua et al. found that common clinical parameters, including BMI, bore minimal relationship to the baseline level of sperm DNA fragmentation in an infertile cohort (13). This apparent discrepancy is resolved by our results, which demonstrate that while BMI may be a weak correlate of static DNA damage in a cross-sectional snapshot, it emerges as a powerful predictor of dynamic, cryopreservation-induced DNA damage. This highlights a critical interaction wherein the metabolic state of the individual, reflected by BMI, significantly modulates cellular resilience to the acute oxidative and physical stresses of the freeze-thaw process. Our study therefore bridges observational correlations and functional outcome, identifying BMI not merely as a marker of poorer semen quality, but as a specific risk factor for iatrogenic damage during fertility preservation.

The protective effect of better sperm morphology likely reflects superior inherent chromatin compaction and structural integrity of the sperm head, conferring resilience against the physical and oxidative stresses of freezing (15). Notably, while lower sperm count showed a significant negative correlation with ΔDFI in univariate analysis, it was not retained in the final multivariate model. This suggests that in men with severe OAT, sperm quality (morphology) is a more critical determinant of cryo-resilience than sperm quantity (count). The lack of a significant independent association with smoking status in our model aligns with broader literature showing inconsistent effects and suggests that the acute, substantial insult of cryopreservation may overshadow the contribution of this chronic exposure in this specific context (16, 17).

The identification of BMI and sperm morphology as independent predictors of cryopreservation-induced DNA damage provides a foundation for a risk-stratified

approach to fertility preservation in men with severe OAT. Patients presenting with elevated BMI and impaired morphology constitute a recognizable high-risk subgroup for whom tailored clinical and laboratory strategies should be considered to mitigate iatrogenic injury. Given the established association between obesity, oxidative stress, and sperm chromatin vulnerability, a period of targeted antioxidant supplementation prior to semen banking may represent a prudent, though prospectively unvalidated intervention. Furthermore, the application of advanced sperm selection techniques prior to cryopreservation – such as *magnetic-activated cell sorting* (MACS) or density gradient centrifugation – could be prioritized for this cohort to isolate a sperm subpopulation with superior inherent chromatin integrity and membrane stability, thereby potentially enhancing post-thaw resilience. Concurrently, there is a clear need for dedicated comparative studies to evaluate the efficacy of alternative cryoprotectant agents, slow-freezing versus verification protocols, and antioxidant-supplemented freezing media specifically within this high-risk population. Finally, following thawing, the utilization of advanced sperm selection modalities, including *intracytoplasmic morphologically selected sperm injection* (IMSI) or sperm chromatin dispersion assays, may aid in identifying viable spermatozoa with minimal DNA damage for use in subsequent *intracytoplasmic sperm injection* (ICSI) cycles.

Conclusions

In a large cohort of men with severe OAT, cryopreservation consistently and significantly exacerbates sperm DNA fragmentation. Crucially, this effect is not uniform and is powerfully predicted by patient-specific factors. Higher BMI and poorer sperm morphology independently identify a vulnerable patient subgroup at risk for the greatest cryopreservation-induced DNA damage.
This underscores that metabolic health, reflected by BMI, is a key modulator of sperm resilience to freezing stress, beyond its known association with baseline semen quality. Integrating these predictors into clinical practice enables a personalized approach to fertility preservation counseling and laboratory strategy, aiming to optimize the potential of cryopreserved specimens for this challenging population.

Declarations

Ethical approval and consent for participate: *According to the policies of Alexandria University and applicable national regulations, this research did not constitute human subjects research requiring formal Institutional Review Board (IRB) approval. Patient consent for the use of de-identified data in research was obtained per standard institutional protocol at the time of sample collection.*

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