

Original Article

Asian Pacific Journal of Reproduction

Journal homepage: www.apjr.net



doi: 10.4103/apjr.apjr_42_23

The relationship between DNA fragmentation and the intensity of morphologically abnormal human spermatozoa

Mercedes González–Martínez¹, Pascual Sánchez–Martín¹, Carmen López–Fernández², Stephen D. Johnston^{3,4✉}, Jaime Gosálvez²¹*Clínica Ginemed, Unidad de Reproducción, Sevilla, Spain*²*Department of Biology, Universidad Autónoma de Madrid, Cantoblanco, Madrid, Spain*³*School of Environment, The University of Queensland, Gatton, Australia*⁴*School of Veterinary Science, The University of Queensland, Gatton, Australia*

ABSTRACT

Objective: To determine the relationship between teratozoospermia and sperm DNA fragmentation (SDF) in the human ejaculate.

Methods: This retrospective study included 100 normozoospermic men as a control cohort (abnormal forms >14%), 210 patients with a high level of abnormal forms ($\leq 4\%$) and 65 patients presenting with a moderate level of abnormal forms ($>4\%$ to $\leq 14\%$) based on the World Health Organization definitions. Sperm morphology was assessed using bright field microscopy. Sperm DNA fragmentation was assessed using the sperm chromatin dispersion assay. Non-parametric analyses were conducted to examine the relationship between abnormal sperm morphology and sperm DNA fragmentation; receiver operating characteristic (ROC) analyses were conducted to assess sensitivity and specificity of this relationship.

Results: A correlation analysis revealed that the higher the proportion of abnormal spermatozoa in the ejaculate, the higher the level of SDF (Spearman's Rho = -0.230 ; $P < 0.001$). Significant differences in the proportion of SDF were found when all cohorts were compared ($P < 0.001$); these significant differences were also retained when the different cohorts were compared pairwise. ROC analysis showed a moderate but significant predictive value for SDF to differentiate patients with different levels of teratozoospermia.

Conclusions: Although analysis of a more continuous range of values for teratozoospermia would help further clarify any causal relationship with SDF, there is clearly a synergistic or coincident affiliation between these variables that needs to be acknowledged by the clinician when interpreting the spermogram.

KEYWORDS: Teratozoospermia; Sperm morphology; Sperm DNA fragmentation; Male factor; Human reproduction; Human fertility

1. Introduction

Teratozoospermia has been defined as the presence of morphologically abnormal spermatozoa in the ejaculate, most likely resulting from defective cellular differentiation during spermiogenesis. The specific origins of such sperm cell abnormalities are typically obscure but have been linked to variables such as environmental contaminants and stressors, patient age and/or genetic driven factors[1–4]. A lack of proper sperm chromatin maturation during spermiogenesis, may also have direct consequences on chromatin stability and DNA damage[5].

Significance

The direct relationship between teratozoospermia and sperm DNA fragmentation in the human ejaculate is unclear. The results of this study have demonstrated that men with high levels of abnormal sperm morphology in their ejaculate, also appear to have a higher risk of sperm DNA damage. A synergistic affiliation exists between the intensity of teratozoospermia and sperm DNA fragmentation in the human ejaculate. This finding has clinical consequences for the interpretation of the spermogram, the underlying aetiology of the pathology and diagnosis of patient fertility.

✉To whom correspondence may be addressed. E-mail: s.johnston1@uq.edu.au

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, tweak and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2024 Asian Pacific Journal of Reproduction Produced by Wolters Kluwer- Medknow.

How to cite this article: González-Martínez M, Sánchez-Martín P, López-Fernández C, Johnston SD, Gosálvez J. The relationship between DNA fragmentation and the intensity of morphologically abnormal human spermatozoa. *Asian Pac J Reprod* 2024; 13(1): 22-27.

Article history: Received: 21 March 2023; Revision: 29 June 2023; Accepted: 19 October 2023; Available online: 26 January 2024

Consequently, as the majority of abnormal sperm morphologies have been associated with spermiogenic dysfunction, it is not surprising that both phenomena can coexist when measured in the ejaculate [6–9]. Although the precise relationship (causal or otherwise) between the incidence of sperm DNA fragmentation (SDF) and sperm morphology remains to be elucidated, from a clinical perspective, the presence of either high levels of abnormal sperm morphology and/or high levels of SDF is clearly indicative of male infertility [10,11].

The aim of the present investigation was to investigate whether there was a direct correlation between an increase in the incidence of an abnormal sperm morphology (teratozoospermia) in the ejaculate and SDF. We have assumed, as the most parsimonious hypothesis, that SDF will increase, as the level of abnormal spermatozoa increases in the ejaculate.

2. Subjects and methods

2.1. Study design, patient cohorts and inclusion criteria

This retrospective study was conducted from 2019 to 2022 and consisted of a normozoospermic control cohort and two experimental cohorts, samples of which were obtained from a Spanish Fertility Clinic located in Seville, Spain and analysed at the Department of Biology, Autonomous University of Madrid, Spain. For the purposes of the present study and in accordance with the standards of the World Health Organization (WHO) [12], the experimental control cohort consisted of 100 normozoospermic men with >14% normal morphological sperm in their ejaculate; the individual level of abnormal forms was not scored, but only whether it was >14%. The experimental cohorts consisted of men presenting with either a high level of abnormal sperm morphology (HLab; $n=218$) characterised by $\leq 4\%$ of normal spermatozoa in their ejaculate or men presenting with a moderate level of abnormal sperm morphology (MLab; $n=65$), characterised by a range of normal spermatozoa from >4% to $\leq 14\%$. These cohort thresholds were also chosen based on the WHO recommendations, which set a lower threshold level of normal sperm in 1999 [12] at 15%, then subsequently reduced this value to 4% in 2021 [13]. In both HLab and MLab cohorts, men presenting with levels of motility higher than 42% and defined as the sum of progressive and non-progressive motility, were included in the study, whereas men with severe asthenozoospermia, oligozoospermia and oligo-astheno-teratozoospermia also defined by the WHO criteria were excluded.

2.2. Semen analysis

Semen samples were collected at the clinic *via* masturbation into sperm safe plastic containers that were kept at room temperature for 30 min to a maximum of 1 h prior to liquefaction. Ejaculates

showing abnormal sperm morphology were assessed under bright field microscopy using a Nikon Eclipse microscope equipped with a high-resolution Nikon 12bits CCD (Nikon DS-Q) and using 40 \times fluorite objective. After semen smears were aird dried, they were fixed in methanol and stained using Diff-Quik (Medion Grifols Diagnostics, Düringen, Switzerland). All samples were analysed using 100 \times immersion oil objectives and 200 spermatozoa were scored. SDF was assessed using the Sperm Chromatin Dispersion test (Halosperm; Halotech DNA, Madrid, Spain) as per the manufacturer's instruction. Samples were assessed for morphology and SDF by the same embryologist (MG-M). SDF was expressed as percentage of sperm showing a fragmented DNA molecule after the observation of 300 spermatozoa.

2.3. Statistical analysis

Statistical analysis was conducted using SPSS (IBM SPSS Statistics Package v26, NY, USA). Data were tested for normal distribution. To compare the values of SDF, all three cohorts were considered as independent variables, and the Kruskal-Wallis H test was used. To compare the values of SDF associated with two independent cohorts, the W Wilcoxon test was employed. Correlation analysis was performed using Spearman test. The predictive values of SDF to differentiate HLab, MLab and control cohorts were calculated using a receiver operating characteristic (ROC) curve analysis based on information provided by the area under the curve (AUC). Standard error and 95% confidence interval (CI) was used in this study.

2.4. Ethics statement

All data included in this study had patient consent and ethical authorisation of both the clinic from which the samples were collected (Clínica Ginemed, Unidad de Reproducción, Sevilla) and the University of Seville (Permit number-3375125c8b5f1d04c9511825aef98d309135328c) human ethics committees. All procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000.

3. Results

3.1. The correlation between teratozoospermia and SDF

To establish an overall general perspective with respect to how the presence of abnormal sperm morphology interacted with SDF, a correlation analysis was first performed using data from individuals of all three cohorts; this analysis revealed a significant negative correlation (Spearman's Rho = -0.363; $P < 0.001$). Given the percentage of normal spermatozoa of the control cohort were only scored by the clinic as having levels of normal sperm >14%, rather than their specific individual level, this represented a limitation on

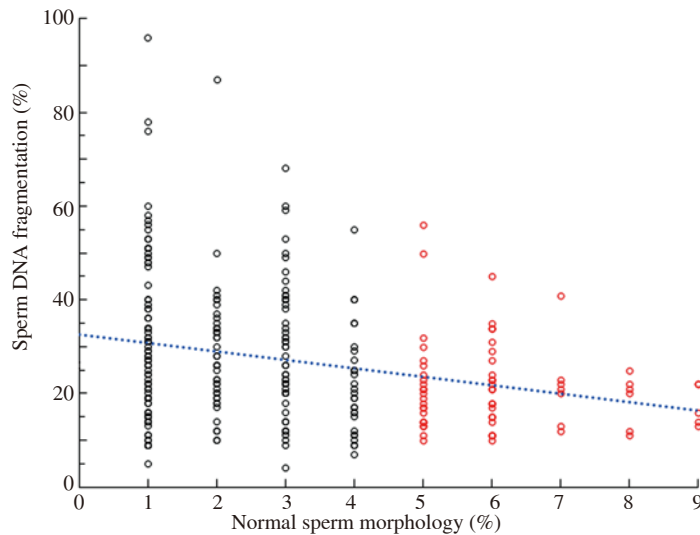


Figure 1. The distribution of sperm DNA fragmentation (SDF) and percentage of normal spermatozoa data observed in individuals with high (HLab: $\leq 4\%$ normal - black circles) and moderate (MLab: $>4\%$ to $<14\%$ - red circles) numbers of abnormal spermatozoa. Blue dotted line - Spearman's Rho = -0.230 ; $P < 0.001$.

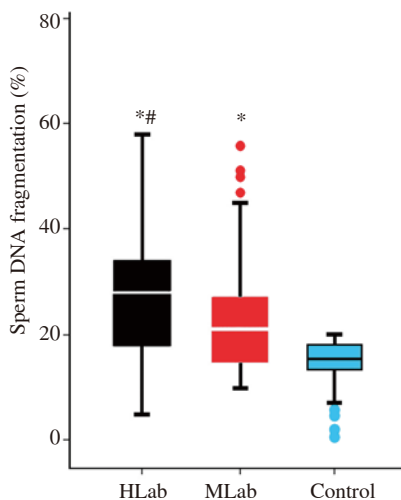


Figure 2. Box and whisker diagram representing the distribution of sperm DNA fragmentation (SDF) observed in males with high (HLab - black) and moderate (MLab - red) levels of abnormal forms and fertile sperm donors (Control - blue). * $P < 0.05$: compared to the control; # $P < 0.05$: compared to MLab.

our correlation analysis and is therefore likely to be understating the strength of any correlation. Consequently, we performed a second correlation analysis which only included the HLab and MLab cohorts which resulted in a weaker but significant negative correlation (Spearman's Rho = -0.230 ; $P < 0.001$) (Figure 1). Together these analyses suggest that when abnormal sperm morphology is considered as a continuous series, the lower the value of normal spermatozoa in the ejaculate, the higher the level of SDF. Figure 2 shows the distribution of the SDF values within each cohort (HLab, MLab and control). Significant differences in the percentage of SDF were found when all cohorts were compared; these differences were also maintained when the different cohorts were compared pairwise.

3.2. Risk prediction with ROC curves

ROC curve analyses were conducted to assess the cut-off values to determine the level of SDF that might be used to differentiate the different levels of teratospermia cohorts (HLab, MLab and control) (Figure 3). Results of the different comparisons are shown in Table 1. P values revealed significant differences between all cohorts except when MLab are compared with the control. The AUC values ranged from 0.58 to 0.70 and were only moderately predictive. In fact, the cut-off values for SDF derived from to each comparison were quite similar, independent of the cohort analysed. Cut-off values and the levels of sensitivity and specificity are showed in Table 1.

4. Discussion

The study has shown that after comparing men with high and moderate levels of morphologically abnormal spermatozoa in their ejaculates, an increasing level of sperm morphological abnormalities was correlated with a corresponding increase in the level of sperm DNA fragmentation, *i.e.*, men with high levels of abnormal sperm morphology in their ejaculates appear to have a higher risk of sperm DNA damage, and *vice-versa*. Statistically significant differences were also found when SDF values observed in men categorised as having moderate (MLab) and high (HLab) levels of morphological alterations were compared with the control sample. While these findings are consistent with results reported elsewhere that demonstrate a strong correlation between the presence of abnormal sperm morphology and sperm DNA quality^[14–16], our study has further revealed that the level of abnormal spermatozoa and SDF show a significant continuous increase with increasing levels of abnormal sperm forms. Consequently, poor reproductive outcomes in

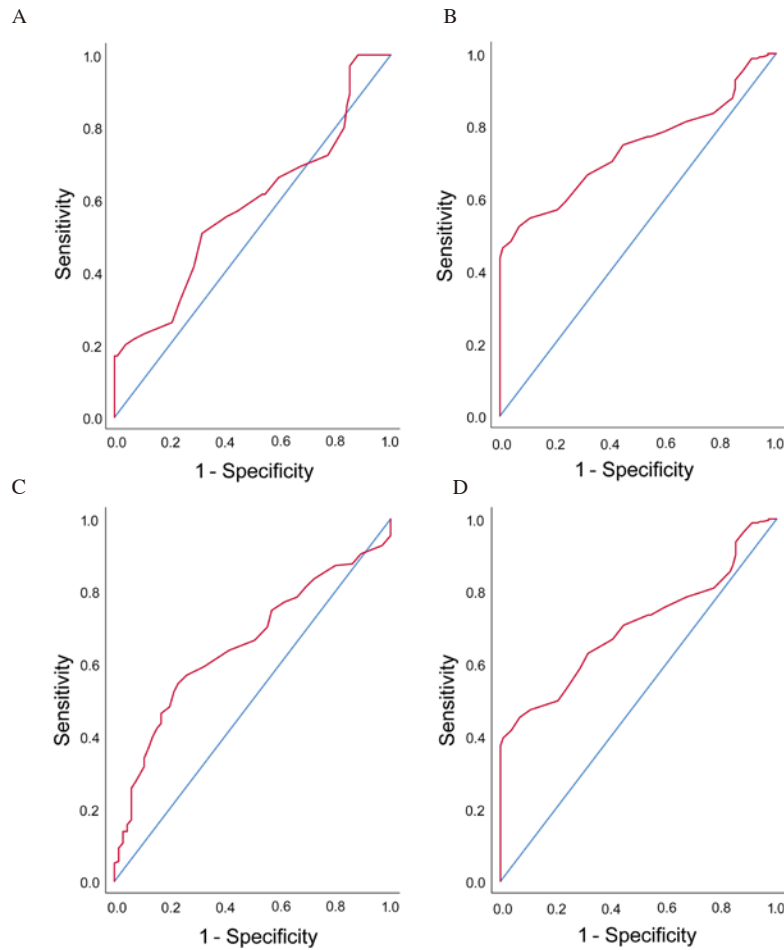


Figure 3. Receiving operator characteristics (ROC) curves to investigate the sensitivity and specificity offered by the level of sperm DNA fragmentation as derived from the comparison of men with (A) moderate level of abnormal forms (MLab) *versus* the control, (B) high level of abnormal forms (HLab) *versus* the control, (C) HLab *versus* MLab of abnormal forms, (D) combined HLab and MLab levels of abnormal forms *versus* the control.

Table 1. Comparative receiving operator characteristics obtained values associated to area under the curve.

Groups	AUC	SD	<i>P</i> value	SDF cut-off	Sensitivity (%)	Specificity (%)
MLab/Control	0.581	0.047	0.083	19.5	55	59
HLab/Control	0.738	0.027	<0.001	20.5	66	69
MLab/HLab	0.654	0.035	<0.001	21.5	63	69
MLab + HLab/Control	0.702	0.026	<0.001	20.5	62	69

HLab: high levels of abnormal forms; MLab: moderate levels of abnormal forms; Control: fertile sperm donors; HLab + MLab: combined high and low levels of abnormal forms. AUC: area under the curve. SD: standard deviation. SDF: sperm DNA fragmentation.

patients with teratozoospermia are not only a consequence of elevated numbers of abnormal spermatozoa but may also be co-dependent on the level of SDF. This finding has clinical consequences for the interpretation of the spermogram, the underlying aetiology and the subsequent diagnosis of the patient's fertility.

The cut-off level of SDF, as revealed by the ROC curve analysis to discriminate individuals with a high level of abnormal forms (HLab) compared to that of the fertile men (control), resulted in an AUC of 0.738 with an SDF discrimination value for SDF between both cohorts of 20.5%; these values are very similar to those previously reported[17] (AUC = 0.746 and SDF discriminatory value for SDF = 18%). While the AUC in both studies might be regarded as moderate, an identical tendency nevertheless exists for an increased level of

abnormal forms when individuals are classified as presenting with an altered level of SDF.

While the severity of sperm abnormalities and their relationship with SDF have not been fully investigated in humans, there are some examples in animal models[18]. In the case of Holstein bulls, two different forms of sperm abnormalities have been defined and classified as being either major or minor sperm abnormalities[19]. Descriptions of the different morphological variations have been summarised by Enciso *et al*[20] who showed that the major sperm abnormalities were associated with a mean SDF value of (85.0±5.0)%, whereas the minor sperm abnormalities showed a significantly lower mean value of (17.8±5.5)%. These observations suggest the same tendency as described in the current study,

where not only the severity of the abnormality, but also the type of morphological variation may influence the SDF. In addition, in the case of Holstein bulls, Sailer *et al*[21] showed that sperm head morphology was not only related to the severity of SDF but also to fertility. Further studies that utilise data that more precisely defines the type of sperm morphology in humans are needed to better evaluate their impact on reproductive outcomes and their relationship with SDF. This will require the andrologist to conduct a more detailed analysis of sperm morphology in each patient.

The close relationship between sperm morphology and chromatin integrity suggests a strong, yet undefined biological relationship between these two parameters. Teratozoospermia is related to the level of aneuploidy and aneuploidy in SDF, which tends to be higher in aneuploid spermatozoa[22]. Therefore, it is possible that some of the detected fragmented DNA may be related to the presence of variable levels of sperm aneuploidy in the ejaculate[23–25]. Muriel *et al*[22] used three-color fluorescence in situ hybridization experiments that targeted chromosome X, Y and 18 and was performed on slides that were pre-processed using the sperm chromatin dispersion assay to identify spermatozoa with and without fragmented DNA. These authors found that spermatozoa with fragmented DNA present with a 4.4× fold increase in the rate of diploidy and a 5× fold increase in disomy with respect to sperm free of SDF. The overall aneuploidy rate was 4.6× fold higher in spermatozoa with fragmented DNA. Additionally, a higher level of sperm DNA damage was associated with sperm presenting aneuploid sex chromosomes produced during first and second meiotic divisions. In comparison to the findings of Muriel *et al*[22], the observed increase of SDF in morphologically abnormal spermatozoa observed in the current study, may indicate, as Muriel and co-workers state, “that the occurrence of these morpho-anomalies during sperm maturation may lead to sperm DNA fragmentation as part of a genomic screening mechanism developed to genetically inactivate sperm with a defective genomic makeup.”

Interestingly, the susceptibility of morphologically abnormal sperm to DNA damage when teratozoospermic patients are cryopreserved and thawed was found to be significantly higher than that observed in ejaculates that contained morphologically normal spermatozoa[26]. This finding suggests that defective protamination and chromatin compaction may produce a low level of DNA protection during physiochemical trauma associated with sperm cell cryopreservation, a phenomenon that is essentially congruent with compromised sperm maturation. Additionally, it is feasible that sperm DNA with deficient protamination is more susceptible to reactive oxygen species damage. In fact, it has been shown that poor quality spermatozoa containing partially decondensed chromatin are more susceptible to DNA damage[27].

Finally, it should be noted that teratozoospermia is frequently present in combination with other sperm alterations, such as those with asthenozoospermia, and oligozoospermia (oligo-asthenoteratozoospermic men), so that full elimination of these factors during sperm evaluation is difficult to control, especially if they

are present at sub-detectable levels. While this possible interactive synergy of differing pathologies needs to be acknowledged as a limitation in the interpretation of our findings, results presented in the current study reinforce the idea that complex and possible interactive or synergistic scenarios, in this case teratozoospermia and SDF, can affect male fertility, such that the ultimate influence of a single abnormality on reproductive outcome is difficult to ascertain.

Conflict of interest statement

The authors declare no conflicts of interest.

Funding

This study received no extramural funding.

Authors' contributions

Mercedes González-Martínez performed sperm DNA fragmentation analysis and data searching. Pascual Sánchez-Martín carried out patient recruitment and study design. Carmen López-Fernández performed sperm DNA fragmentation analysis. Stephen D. Johnston was responsible for data interpretation, draft and final manuscript writing. Jaime Gosálvez performed study design, data analysis and first draft production.

References

- [1] De Braekeleer M, Nguyen MH, Morel F, Perrin A. Genetic aspects of monomorphic teratozoospermia: A review. *J Assist Reprod Genet* 2015; **32**: 615-623. doi: 10.1007/s10815-015-0433-2.
- [2] Danis RB, Samplaski MK. Sperm morphology: History, challenges, and impact on natural and assisted fertility. *Curr Urol Rep* 2019; **20**: 43. doi: 10.1007/s11934-019-0911-7.
- [3] Franken DR. How accurate is sperm morphology as an indicator of sperm function? *Andrologia* 2015; **47**: 720-723. doi: 10.1111/and.12324.
- [4] Linn E, Ghanem L, Bhakta H, Greer C, Avella M. Genes regulating spermatogenesis and sperm function associated with rare disorders. *Front Cell Dev Biol* 2021; **9**: 634536. doi: 10.3389/fcell.2021.634536.
- [5] Sivanarayana T, Krishna ChR, Prakash GJ, Krishna KM, Madan K, Rani BS, et al. CASA derived human sperm abnormalities: Correlation with chromatin packing and DNA fragmentation. *J Assist Reprod Genet* 2012; **29**: 1327-1334. doi: 10.1007/s10815-012-9885-9.
- [6] Daris B, Goropevsek A, Hojnik N, Vlaisavljevi V. Sperm morphological abnormalities as indicators of DNA fragmentation and fertilization in ICSI. *Arch Gynecol Obstet* 2010; **281**: 363-367. doi: 10.1007/s00404-009-1140-y.

- [7] Mangiarini A, Paffoni A, Restelli L, Ferrari S, Guarneri C, Ragni G, et al. Specific sperm defects are differentially correlated with DNA fragmentation in both normozoospermic and teratozoospermic subjects. *Andrology* 2013; **1**: 838-844. doi: 10.1111/j.2047-2927.2013.00138.x.
- [8] Skowronek F, Casanova G, Alciaturi J, Capurro A, Cantu L, Montes JM, et al. DNA sperm damage correlates with nuclear ultrastructural sperm defects in teratozoospermic men. *Andrologia* 2012; **44**: 59-65. doi: 10.1111/j.1439-0272.2010.01106.x.
- [9] Candela L, Boeri L, Capogrosso P, Cazzaniga W, Pozzi E, Belladelli F, et al. Correlation among isolated teratozoospermia, sperm DNA fragmentation and markers of systemic inflammation in primary infertile men. *PLoS One* 2021; **16**(6): e0251608. doi: 10.1371/journal.pone.0251608.
- [10] Szczygiel M, Kurpisz M. Teratozoospermia and its effect on male fertility potential. *Andrologia* 1999; **31**: 63-75.
- [11] Hotaling JM, Smith JF, Rosen M, Muller CH, Walsh TJ. The relationship between isolated teratozoospermia and clinical pregnancy after *in vitro* fertilization with or without intracytoplasmic sperm injection: A systematic review and meta-analysis. *Fertil Steril* 2011; **95**(3): 1141-1145. doi: 10.1016/j.fertnstert.2010.09.029.
- [12] World Health Organization. *WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction*. 4th ed. Geneva: World Health Organization; 1999. [Online] Available from <https://www.aab.org/images/WHO%204th%20manual.pdf> [Accessed 7 March 2023].
- [13] World Health Organization. *WHO laboratory manual for the examination and processing of human semen*. 6th ed. World Health Organization; 2021. [Online] Available from <https://apps.who.int/iris/handle/10665/343208> [Accessed 7 March 2023].
- [14] Fischer MA, Willis J, Zini A. Human sperm DNA integrity: Correlation with sperm cytoplasmic droplets. *Urology* 2003; **61**: 207-211. doi: 10.1016/s0090-4295(02)02098-8.
- [15] Khalili MA, Aghaie-Maybodi F, Anvari M, Talebi AR. Sperm nuclear DNA in ejaculates of fertile and infertile men: Correlation with semen parameters. *Urol J* 2006; **3**: 154-159.
- [16] Braham A, Ghedir H, Zidi I, Sallem A, Hajlaoui A, Ajina M, et al. Nuclear sperm quality in total polymorphic teratozoospermia and its impact on intracytoplasmic sperm injection outcome. *Andrologia* 2019; **51**(5): e13252. doi: 10.1111/and.13252.
- [17] Jakubik-Uljasz J, Gill K, Rosiak-Gill A, Piasecka M. Relationship between sperm morphology and sperm DNA dispersion. *Transl Androl Urol* 2020; **9**: 405-415. doi: 10.21037/tau.2020.01.31.
- [18] Siddique RA, Mohanarao GJ, Kumar R, Kumar A, Malik PK, Kumar C, et al. Sperm abnormalities and DNA fragmentation vis-à-vis Mammalian male infertility—A review. *WJAS* 2011; 174-189.
- [19] Blom E. The ultrastructure of some characteristic sperm defects and a proposal for a new classification of the bull spermogram. *Nord Vet Med* 1973; **25**: 383-391.
- [20] Enciso M, Cisale H, Johnston SD, Sarasa J, Fernández JL, Gosálvez J. Major morphological sperm abnormalities in the bull are related to sperm DNA damage. *Theriogenology* 2011; **76**: 23-32. doi: 10.1016/j.theriogenology.2010.12.034.
- [21] Sailer BL, Jost LK, Evenson DP. Bull sperm head morphometry related to abnormal chromatin structure and fertility. *Cytometry* 1996; **24**(2): 167-173.
- [22] Muriel L, Goyanes V, Segrelles E, Gosálvez J, Álvarez JG, Fernández JL. Increased aneuploidy rate in sperm with fragmented DNA as determined by the sperm chromatin dispersion (SCD) test and FISH analysis. *J Androl* 2007; **28**: 38-49. doi: 10.2164/jandrol.106.000067.
- [23] Rives N, Saint Clair A, Mazurier S, Sibert L, Simeon N, Joly G, et al. Relationship between clinical phenotype, semen parameters and aneuploidy frequency in sperm nuclei of 50 infertile males. *Hum Genet* 1999; **105**: 266-272. doi: 10.1007/s004390051100.
- [24] Lewis-Jones I, Aziz N, Seshadri S, Douglas A, Howard P. Sperm chromosomal abnormalities are linked to sperm morphologic deformities. *Fertil Steril* 2003; **79**: 212-215. doi: 10.1016/s0015-0282(02)04411-4.
- [25] Calogero AE, De Palma A, Grazioso C, Barone N, Romeo R, Rappazzo G, et al. Aneuploidy rate in spermatozoa of selected men with abnormal semen parameters. *Hum Reprod* 2001; **16**: 1172-1179. doi: 10.1093/humrep/16.6.1172.
- [26] Kalthur G, Adiga SK, Upadhy D, Rao S, Kumar P. Effect of cryopreservation on sperm DNA integrity in patients with teratospermia. *Fertil Steril* 2008; **89**: 1723-1727. doi: 10.1016/j.fertnstert.2007.06.087.
- [27] Gorczyca W, Traganos F, Jesionowska H, Darzynkiewicz Z. Presence of DNA strand breaks and increased sensitivity of DNA *in situ* to denaturation in abnormal human sperm cells: Analogy to apoptosis of somatic cells. *Exp Cell Res* 1993; **207**: 202-205. doi: 10.1006/excr.1993.1182.

Publisher's Note

The Publisher of the *Journal* remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Edited by Lin LY, Lei Y