ORIGINAL RESEARCH ARTICLE

Correlation of sperm DNA fragmentation index with routine semen parameters and its predictive value for clinical outcomes of in vitro fertilization

DOI: 10.29063/ajrh2026/v30i3.8

*Shijie Bi, Qingfeng Jiang, Xi Yang, Lichun Zhao, Qingxiang Wang, Xiaohui Chen, Kailiang Ji, Wenyue Zhao and Lijuan He**

Department of Reproductive Medicine; The People's Hospital of Chuxiong Yi Autonomous Prefecture; Chuxiong 675000, Yunnan Province, China

***For Correspondence**: Email**:** *heljphcyap@hosp-edu.cn*

Abstract

Infertility refers to a couple's inability to conceive after a period of normal, unprotected sexual activity or to carry a pregnancy to full term. This study investigated the correlation between sperm DNA fragmentation index (DFI) and routine semen parameters, as well as its predictive value for in vitro fertilization (IVF) outcomes. A total of 158 infertile patients undergoing IVF-embryo transfer (ET) from September 2021 to February 2023 were enrolled. Based on DFI levels, patients were divided into a low-DFI group (DFI ≤ 25%, n=94) and a high-DFI group (DFI > 25%, n=64). Routine semen parameters were analyzed and correlated with DFI levels. DFI showed significant negative correlations with sperm motility, progressive motility (PR), and non-progressive motility (NP), and a positive correlation with immotility (IM) (P<0.05).Receiver operating characteristic (ROC) analysis revealed that combined semen parameters had the highest diagnostic value for assessing DFI (AUC = 0.998). Additionally, significant differences were observed between groups in human chorionic gonadotropin (hCG) positivity and clinical pregnancy rates (P<0.05). We conclude that sperm DFI is negatively associated with sperm motility parameters and positively associated with immotility. .It has a predictive value for clinical IVF outcomes and may serve as a useful indicator in infertility assessment. *(Afr J Reprod Health 2026; 30 [3]:95 -103).*

Keywords: DNA; fragmentation; in vitro fertilization; semen; sperm

Résumé

L'infertilité désigne l'incapacité d'un couple à concevoir après une période d'activité sexuelle normale et non protégée ou à mener une grossesse à terme. Cette étude a examiné la corrélation entre l'indice de fragmentation de l'adn du sperme (idd) et les paramètres de routine du sperme, ainsi que sa valeur prédictive pour les résultats de la fécondation in vitro (fiv). Un total de 158 patients infertiles ayant subi un transfert d'embryon fiv (ET) de septembre 2021 à février 2023 ont été recrutés. D'après les taux d'idd, les patients ont été divisés en un groupe à faible idd (DFI ≤ 25%, n=94) et un groupe à haut DFI (DFI > 25%, n=64). Les paramètres de Routine du sperme ont été analysés et mis en corrélation avec les niveaux d'idf. Les DFI ont montré des corrélations négatives significatives avec la motilité des spermatozoïdes, la motilité progressive (PR) et la motilité non progressive (NP), et une corrélation positive avec l'immotilité (IM) (P< 0,05). L'analyse des caractéristiques de fonctionnement du récepteur (ROC) a révélé que les paramètres combinés du sperme avaient la valeur diagnostique la plus élevée pour évaluer la DFI (AUC = 0,998). De plus, des différences significatives ont été observées entre les groupes ence qui concerne la positivité de la gonadotrophine chorionique humaine (hCG) et les taux de grossesse clinique (P< 0,05). Nous concluons que la DFI du sperme est négativement associée aux paramètres de motilité des spermatozoïdes et positivement associée à l'immotilité. Il a une valeur prédictive pour les résultats cliniques de la fiv et peut servir d'indicateur utile dans l'évaluation de l'infertilité. *(Afr J Reprod Health 2026; 30 [3]: 95-103).*

Mots-clés: adn; La fragmentation; La fécondation in vitro; Le sperme; Le sperme

Introduction

Infertility is defined as the inability to conceive or to carry a pregnancy to term despite regular unprotected intercourse.[1]In addition to female factors (such as ovulatory dysfunction and uterine abnormalities, endocrine disorders) and male factors (such as sperm quality problems and sexual dysfunction), infertility may also be attributed to immunological factors, genetic factors, unhealthy lifestyle, and adverse environmental factors[2,3]. Currently, infertility has been considered the third

largest category of diseases affecting human health, also as one of the major global public health and clinical issues, and about 40% of infertility cases are ascribed to male factors[4]. The assessment methods for male fertility have changed from detection of the morphology, quantity, and sperm concentration in the past to in-depth exploration at the cellular and molecular levels at present. Sperm DNA fragmentation index (DFI) refers to the proportion of DNA with single-strand breaks to the total sperm DNA, which is a crucial indicator reflecting the degree of sperm DNA fragmentation (SDF), enabling more accurate evaluation of sperm quality microscopically and genetically[5]. The level of DFI plays a crucial role in male fertility. For example, an excessively high DFI level possibly suggests decreased quality of sperms, which enhances the difficulties in conception[6]. In vitro fertilization-embryo transfer (IVF-ET) technology exerts obvious therapeutic effects on infertility, through which the fusion of ova with sperm is realized in an external laboratory environment, and then the formed embryo is transferred back into the womb of females[7]. In practice, however, pregnancy failure and recurrent miscarriage are still found in some patients receiving IVF-ET treatment. Given this, deeply exploring the relationship between sperm DFI and routine semen parameters as well as its predictive value for the clinical outcomes of IVF is of great clinical significance.

In the present study, sperm DFI was detected, and its correlations with routine semen parameters including sperm motility rate, progressive motility (PR) rate, non-progressive motility (NP) rate and immotility (IM) rate were determined. The aim of this study was to provide a reliable reference for predicting the clinical outcomes of IVF.

Methods

Subjects

Subjects (n=158) were selected from men with infertility who received outpatient treatment at The People's Hospital of Chuxiong Yi Autonomous Prefecture between September 2021 and February 2023. Based on their sperm DNA fragmentation index (DFI), patients were categorized into a low-DFI group (DFI ≤ 25%, n=94) and a high-DFI group (DFI >25%, n=64) according to the grouping method described in previous literature[8].

Inclusion and exclusion criteria

The inclusion criteria were as follows: 1) males aged 20-45 years undergoing routine semen tests; 2) those who had cohabited with their spouses for 1 year or more, with regular sexual activity (minimum frequency of once per week); 3) those without a history of testicular or epididymal trauma; 4) those without a history of varicocele or having undergone corrective surgery at least 6 months prior to enrollment; 5) those without hormonal therapy use within the last 6 months; 6) those without comorbidities that may directly impact fertility.

The exclusion criteria involved: 1) males with infertility due to confirmed female factors (such as fallopian tube obstruction and ovulatory dysfunction) or their spouses not suitable for conception owing to malignant diseases; 2) those with acute reproductive system infections, familial-hereditary diseases, or chromosomal abnormalities; 3) those currently using medications known to negatively impact sperm DNA integrity (e.g. certain chemotherapeutic agents).

Sperm quality test

Following 2-7 days of sexual abstinence in males, the urethral orifice and genitals were routinely disinfected, and then the semen was collected through masturbation and placed in a clean and sterilized container to avoid contamination and leakage. Next, a portion of the acquired semen was liquified and then subjected to routine semen analysis and SDF determination[9], and the remaining was utilized for IVF treatment. Routine examinations on the semen of all patients were conducted using the Sperm Class Analyzer (Microptic, Barcelona, Spain).

Sperm DNA integrity test

The sperm chromatin dispersion test was conducted to determine sperm DFI[10]. In detail, the test was performed using Halosperm® kit (Halotech DNA, Madrid, Spain) to assess sperm DNA fragmentation. Sperm samples were processed by embedding cells in low-melting-point agarose on slides, followed by acidic denaturation and lysis to remove nuclear proteins. After staining, they were examined under a microscope, with intact DNA forming halos around the nuclei, while fragmented DNA exhibited

minimal or no halo. DFI was calculated based on halo patterns.

IVF treatment

Semen samples were subjected to density gradient centrifugation with 80% and 40% silanized silica gel suspensions. In detail, 1-2 mL of semen was layered over the gradients, with 80% at the bottom and 40% above it, followed by centrifugation at 300 ×g for 20 minutes at room temperature. This process effectively separated motile, morphologically normal sperm, which concentrated at the bottom layer, from debris and less viable sperm, which remained in the upper layers. The sperm pellet was then collected for further analysis. After washing, the upper liquid was discarded, and the remaining liquid was centrifuged and washed again. Next, the pellet was resuspended gently before being used for assisted reproduction, and 0.5 mL of the liquid was harvested for the assisted reproductive technology. Routine ovulation induction was completed (the mini-stimulation protocol was employed in a small number of subjects due to the decline of the females' ovarian function[11]) based on the condition of spouses, and the follicle was continuously monitored with B-ultrasound examination. When the dominant follicle matured (with a diameter of 16-22 mm), 5000-7500 IU of human chorionic gonadotrophin (HCG) was intramuscularly injected for ovulation, after which conventional luteal support was given.

Pregnancy outcome determination

Two weeks after embryo transfer, blood β-HCG was measured to determine whether biochemical pregnancy was established. Thirty days after transfer, B-ultrasound examination was performed, and clinical pregnancy was confirmed when there was a gestational sac or a fetal heart beat in the uterine cavity. Spontaneous abortion within 12 weeks after pregnancy was considered as first-trimester abortion.

Statistical analysis

Statistical analysis was performed with statistical software SPSS22.0 (IBM Inc., Armonk, USA). Measurement data were normally distributed as confirmed by the Shapiro-Wilk test, and expressed as mean ± standard deviation ($x \pm s$). They were subjected to the *t*-test for pairwise comparison between two groups and one-way analysis of variance for mean comparison among multiple groups. Pearson correlation analysis was conducted on routine semen parameters.

The receiver operating characteristic (ROC) curves were plotted, based on which the predictive value of DFI for the clinical outcomes of IVF was evaluated. $P<0.05$ denoted that the difference was statistically significant.

Ethical considerations

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of The People's Hospital of Chuxiong Yi Autonomous Prefecture. As this was a retrospective analysis of anonymized clinical data, the committee determined that informed consent could be waived

Results

General data of patients

The age of males, age of females, body mass index of females, years of infertility, basal follicle stimulating hormone level, and number of fertilized oocytes showed no differences between the two groups ($P>0.05$) (Table 1).

Results of differential analysis on routine semen parameters.

The results of differential analysis on routine semen parameters showed that the sperm concentration showed no significant difference between low-DFI and high-DFI groups ($P>0.05$), but the sperm motility rate, PR rate, NP rate and IM rate varied between the two groups ($P<0.05$) (Table 2).

Results of correlation analysis between DFI and routine semen parameters

The routine semen parameters sperm motility rate, PR rate and NP rate had significant positive correlations with low DFI level, whereas IM rate displayed a significant negative correlation with low DFI level ($P<0.05$) (Table 3).

Table 1: General data of patients ($x \pm s$)

Item	Low-DFI group (n=94)	High-DFI group (n=64)	*t*	P
Age of males (year)	31.56±2.31	32.14±2.23	1.571	0.118
Age of females (year)	29.44±1.33	29.63±1.61	0.809	0.420
Body mass index of females (kg/m^2)	20.32±2.31	20.54±1.96	0.624	0.534
Years of infertility	3.44±0.51	3.49±0.56	0.581	0.561
Basal follicle stimulating hormone level (U/L)	9.85±1.24	9.68±1.31	0.827	0.409
Quantity of fertilized oocytes	8.21±1.21	8.02±1.08	1.011	0.313

DFI: DNA fragmentation index.

Table 2: Differential analysis on routine semen parameters

Item	Low-DFI group (n=94)	High-DFI group (n=64)	t	P
Sperm concentration (×10^7/mL)	2.45±0.32	2.48±0.54	0.438	0.662
Sperm motility rate	59.44±7.21	31.25±5.33	26.694	0.001
PR rate	35.34±5.78	17.23±6.56	18.299	0.001
NP rate	21.28±7.21	16.87±6.24	3.981	0.001
IM rate	46.24±7.25	58.23±7.68	9.962	0.001

DFI: DNA fragmentation index; IM: immotility; NP: non-progressive motility; PR: progressive motility.

Table 3: Results of correlation analysis between DFI and routine semen parameters

		Sperm motility rate	PR rate	NP rate	IM rate
DFI	**Pearson correlation**	-0.906*	-0.826*	-0.304*	0.623*
	P	0.001	0.001	0.001	0.001

DFI: DNA fragmentation index; IM: immotility; NP: non-progressive motility; PR: progressive motility. *Correlation is significant at the 0.01 level

Assessment value of routine semen parameters for DFI

ROC curves were plotted with sensitivity as the Y-axis and "1-specificity" as the X-axis, with the level of DFI as the dependent variable (low DFI=1, high DFI=0) and sperm motility rate, PR rate, NP rate, IM rate, and their combination as independent variables (Figure 1). It was found that the areas under the curves (AUC) of sperm motility rate, PR rate, NP rate, and IM rate for assessing DFI were 0.995, 0.982, 0.682 and 0.870, respectively, denoting that the above indicators exhibited high values for DFI assessment. The AUC value of their combination was 0.998, exceeding those of single indicators, indicating that their combination was superior to single indicators for assessing DFI (Table 4).

Differences in pregnancy outcomes between DFI groups

The number of HCG-positive cases and clinical pregnancy rate displayed significant differences between the two groups (P<0.05), while the first-trimester abortion rate was of no significant difference between the two groups (P>0.05) (Table 5).

Table 4: Assessment value of routine semen parameters for DFI

Factor	Area under the curve	Cut-off value	95% confidence interval	P	Specificity	Sensitivity	Youden index
Sperm motility rate	0.995	0.006	0.984-1.000	0.001	0.484	0.989	0.473
PR rate	0.982	0.008	0.966-0.997	0.001	0.781	0.968	0.749
NP rate	0.682	0.043	0.598-0.767	0.001	0.203	0.904	0.107
IM rate	0.870	0.03	0.810-0.929	0.001	0.312	0.979	0.291
Combination	0.998	0.002	0.994-1.000	0.001	0.937	0.989	0.926

DFI: DNA fragmentation index; IM: immotility; NP: non-progressive motility; PR: progressive motility.

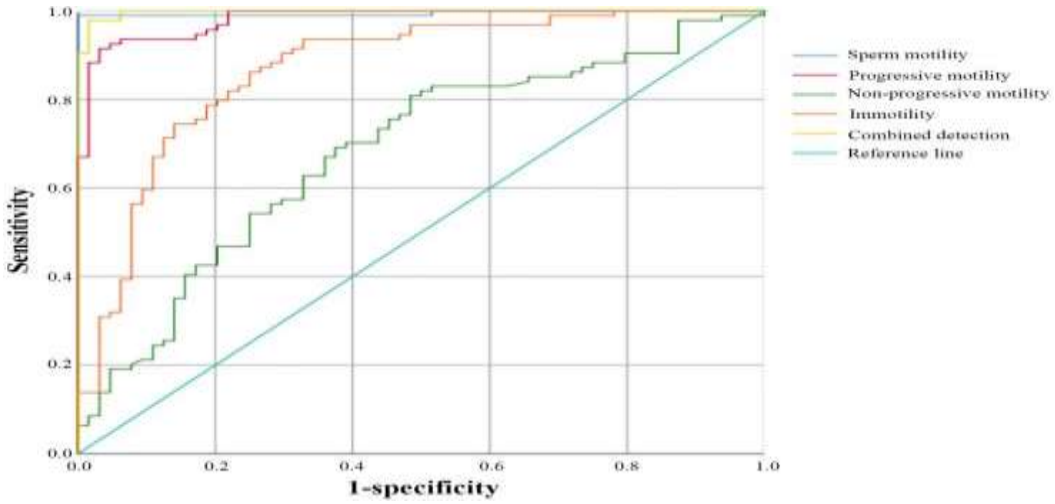

Figure 1: ROC curves of assessment value of routine semen parameters for DFI. DFI: DNA fragmentation index; ROC: receiver operating characteristic.

Table 5: Differences in pregnancy outcomes between DFI groups

Group	Number of HCG-positive cases	Clinical pregnancy rate	First-trimester abortion rate
Low-DFI (n=94)	48 (51.06)	42 (44.68)	9 (9.57)
High-DFI (n=64)	20 (31.25)	18 (28.13)	5 (7.81)
Statistical value	48.035	51.571	0.123
P	0.001	0.001	0.725

DFI: DNA fragmentation index; HCG: human chorionic gonadotrophin.

Table 6: Predictive value of DFI for IVF outcomes

	Area under the curve	Cut-off value	95% confidence interval	Specificity	Sensitivity	Youden index
DFI	0.585	0.046	0.494-0.676	0.469	0.7	0.169

DFI: DNA fragmentation index; IVF: in vitro fertilization

Predictive value of DFI for clinical pregnancy

ROC curve analysis was conducted with clinical pregnancy as the dependent variable (success=1, failure=0), DFI as the independent variable, sensitivity as the Y-axis, and "1-specificity" as the X-axis (Figure 2). The results revealed that the AUC value of DFI for assessing clinical pregnancy was 0.585, indicating that DFI had a high predictive value for clinical pregnancy (Table 6).

Discussion

Male factors alone contribute to about 40% of infertility in couples, and directly cause 1-10% of sterility[12]. Male reproduction mainly involves steps such as sperm generation, maturation, and excretion, and reproductive dysfunction occurs if any of these steps is interfered with or affected by diseases or other factors[13]. Routine semen analysis has long been applied to assess the major indicators of male fertility. However, there are drastic fluctuations in the results of routine semen analysis, which are attributable to various factors including the period of sexual abstinence, physical condition, and subjective emotions, and sometimes the results cannot reflect the actual fertility level of patients, influencing the formulation of therapeutic regimens [14]. Hence, routine semen analysis is not necessarily the optimal approach for male fertility analysis[15]. SDF is prevalent in infertile males, and sperm DNA loss has been generally considered from the aspects of oxidative stress, abnormal sperm apoptosis, and abnormal sperm chromatin assembly[16-18].

Recently, sperm DFI test has been successively used in large reproductive centers in China and other countries for predicting the outcomes of IVF-ET[19,20]. Sperm DFI has been proven to have negative influences on male fertility at present[21]. Besides, sperm DFI is associated with poor embryo development, low implantation rate, and high abortion rate after IVF treatment[22]. Nevertheless, some scholars reported that sperm DFI level has no correlation with the clinical outcomes of IVF[23,24].

In the present study, the general data of 158 patients were compared, and no intergroup differences were observed. Besides, the sperm concentration was not different between the two groups. Probably, sperm generation and maturation are complex physiological processes modulated by multiple links and factors, and although DFI may reflect the integrity of sperm genetic material, sperm concentration mainly hinges on the spermatogenic function of testes and the transportation and storage of sperms, which may be independently modulated by different physiological mechanisms. Therefore, there is no direct correlation between DFI and sperm concentration[25]. Moreover, sperm concentration may be more affected by the quantity of spermatogenic cells, hormone levels, and other factors, which, however, have no close associations with the formation of DNA fragments[26]. We herein found that the high-DFI group displayed decreases in the sperm motility rate, PR rate and NP rate, and increase in the IM rate. Possibly, damaged DNA interfered with the normal function of mitochondria and other organelles, giving rise to an insufficient supply of energy required for sperm motility [27]. DNA fragments may pose an impact on the flagellar structure and function of sperms, causing movement failure of flagella and thus reducing sperm motility, which not only decreases the motility rate but also alters the proportional distribution of sperms in different motility states, including the reduction in NP rate. Damaged DNA may reduce sperm cell membrane stability and affect ion channels and signal transduction, posing a negative impact on sperm motility[28].

In this study, among routine semen parameters, the sperm motility rate, PR rate and NP rate were positively correlated with low DFI level, whereas the IM rate was negatively correlated with low DFI level. A low DFI level suggests high sperm DNA integrity. Sperms with intact DNA usually exhibit more normal physiological functions and smoother energy metabolism, signal transduction and other processes, which provides a strong support for sperm motility. Intact DNA is also conducive to maintaining the stability of sperm structure, thereby raising the sperm motility rate, as well as PR and NP rates accordingly. In most cases, sperms with a low DFI level have higher quality, and during natural selection, high-quality sperms are more likely to remain motile to complete the fertilization process, whereas immobile sperms tend to have various defects, possibly including a higher degree of DNA damage. Besides, sperms with low DFI levels are able to use energy more efficiently for motility. On the contrary, sperms with high DFI levels may

consume considerable energy in the process of responding to DNA damage repair, resulting in decreased motility or even immobility[29].

In the present study, these four routine semen parameters had high evaluation values for DFI, and their combination performed better in evaluating DFI. In addition, significant differences were observed in the number of HCG-positive cases and clinical pregnancy rate between the two groups, but the first-trimester abortion rate was not significantly different between the two groups. The low-DFI group had more HCG-positive cases. Probably, sperms in the low-DFI group have high DNA integrity, so the genetic information carried by them is more stable, and the genetic material can be transmitted to the ovum more accurately during fertilization, thereby elevating the success rate of fertilization. Moreover, higher sperm quality contributes to the formation of healthier embryos, improving the ability of embryos to implant in the uterus and thus elevating the clinical pregnancy rate. However, a high proportion of sperm exhibiting DNA fragmentation was observed in the high-DFI group, which may impair sperm function and fertilization ability. Even in the case of successful fertilization, the poor embryo quality may affect implantation, reducing the number of HCG-positive cases and clinical pregnancy rate [30]. Besides sperm DFI, other factors (such as endocrine status and endometrial receptivity of females, immunological factors and chromosomal abnormalities) may also have evident effects on first-trimester abortion[31]. A difference was herein found in sperm quality between the two groups, but the complexity of other factors may cover up the difference in first-trimester abortion caused by various DFI values. Furthermore, we found that DFI had a high predictive value for clinical pregnancy

Study strengths and limitations

This study has several notable strengths. First, it analyzed a well-defined cohort of infertile men undergoing IVF treatment, enabling a direct assessment of the relationship between sperm DNA fragmentation and clinically relevant reproductive outcomes. Second, the study integrated routine semen parameters with DFI measurements, providing a more comprehensive evaluation of sperm quality that reflects both structural integrity and functional characteristics. Third, the use of standardized laboratory procedures, including the Halosperm® assay and computer-assisted semen analysis, strengthened the reliability of the data.

Nonetheless, several limitations should be acknowledged. As a retrospective single-center study, the findings may be subject to inherent selection bias and may not be fully generalizable to other populations or clinical settings. Additionally, potential confounders such as lifestyle factors, environmental exposures, or underlying comorbidities were not fully captured, which may influence DFI or IVF outcomes. The sample size, although adequate for preliminary associations, may still limit subgroup analyses. Finally, because female factors also contribute significantly to IVF success, unmeasured variations in ovarian reserve, endometrial receptivity, or embryo quality could partly obscure the independent effect of male DFI.
Despite these limitations, the findings have important implications for clinical practice. Incorporating DFI assessment into routine male infertility evaluation may improve risk stratification and counseling for couples undergoing IVF. The results also highlight the need for greater attention to sperm DNA integrity in reproductive medicine and may inform future policy recommendations regarding standardized DFI testing as part of male fertility workup.

Conclusion

In conclusion, the sperm motility rate, PR rate, and NP rate have negative correlations with DFI, while IM rate displays a positive correlation with DFI. Sperm DFI has certain predictive value for the clinical outcomes of IVF. Hence, in clinical practice, particular attention should be paid to sperm DFI test, and sperm DFI, together with routine semen parameters and other indicators, can be utilized for the comprehensive assessment of sperm quality, rendering a basis for the diagnosis and treatment of infertility.

Data availability statement

The data supporting the findings of this study are available upon reasonable request from the corresponding author.

Conflicts of interest

The authors declare no conflict of interest.

Funding

This study was financially supported by the Funded Project of Scientific Research Foundation of Chuxiong Prefecture People's Hospital (No. 2021J24).

Contribution of authors

Shijie Bi and Qingfeng Jiang contributed to the conception and design of the study. Xi Yang, Lichun Zhao, Qingxiang Wang, and Xiaohui Chen were involved in data collection and experimental procedures. Kailiang Ji and Wenyue Zhao performed the data analysis and interpretation. Lijuan He prepared the manuscript and supervised the overall project. All authors reviewed and approved the final version of the manuscript.

References

1. Shacfe G, Turko R, Syed HH, Masoud I, Tahmaz Y, Samhan LM, Alkattan K, Shafqat A and Yaqinuddin A. A DNA Methylation Perspective on Infertility. *Genes* 14, no. 12 (2023): 2132. doi: 10.3390/genes14122132.
2. Q. Sang, P. F. Ray, and L. Wang.Understanding the genetics of human infertility.*Science* 380, no. 6641 (2023): 158-163. doi: 10.1126/science.adf7760.
3. Dougherty MP, Poch AM, Chorich LP, Hawkins ZA, Xu H, Roman RA, Liu H, Brakta S, Taylor HS, Knight J, Kim HG, Diamond MP and Layman LC.Unexplained Female Infertility Associated with Genetic Disease Variants. *New England Journal of Medicine* 388, no. 11 (2023): 1055-1056. doi:10.1056/NEJMc2211539.
4. S. W. Andrabi, A. Ara, A. Saharan, M. Jaffar, N. Gugnani, and S. C. Esteves, "Sperm DNA Fragmentation: causes, evaluation and management in male infertility," *JBRA Assisted Reproduction* 28, no. 2 (2024): 306-319.
5. Osaka A, Okada H, Onozuka S, Tanaka T, Iwahata T, Shimomura Y, Kobori Y, Saito K and Sugimoto K. "Evaluation of the sperm DNA fragmentation index in infertile Japanese men by in-house flow cytometric analysis," *Asian Journal of Andrology* 24, no. 1 (2022): 40-44.
6. H. Yang, G. Li, H. Jin, Y. Guo, and Y. Sun, "The effect of sperm DNA fragmentation index on assisted reproductive technology outcomes and its relationship with semen parameters and lifestyle," *Translational andrology and urology* 8, (2019): 356-365.
7. D. Glujovsky, A. M. Q. Retamar, C. R. A. Sedo, A. Ciapponi, S. Cornelisse, and D. Blake, "Cleavage-stage versus blastocyst-stage embryo transfer in assisted reproductive technology," *Cochrane database of systematic reviews* 5, (2022): CD002118.
8. Wang QX, Wang X, Yu MY, Sun H, Wang D, Zhong SP and Guo F., "Random sperm DNA fragmentation index is not associated with clinical outcomes in day-3 frozen embryo transfer," *Asian journal of andrology* 24, no. 1 (2022): 109-115.
9. S. C. Esteves, "Evolution of the World Health Organization semen analysis manual: where are we?" *Nature Reviews Urology* 19, no. 7 (2022): 439-446.
10. Chatzimeletiou K, Fleva A, Nikolopoulos TT, Markopoulou M, Zervakakou G, Papanikolaou K, Anifandis G, Gianakou A and Grimbizis G. "Evaluation of Sperm DNA Fragmentation Using Two Different Methods: TUNEL via Fluorescence Microscopy, and Flow Cytometry," *Medicina* 59, no. 7 (2023): 1313.doi: 10.3390/medicina59071313.
11. W. Li, and R. C. Chian, "Chinese expert consensus on clinical practice of female fertility preservation," *Reproductive and Developmental Medicine* 6, no. 1 (2022): 42-50.
12. Okonofua FE, Ntoimo LFC, Omonkhua A, Ayodeji O, Olafusi C, Unuabonah E, Ohenhen V., "Causes and risk factors for male infertility: a scoping review of published studies." *International Journal of General Medicine* 15, (2022): 5985-5997.
13. R. Mazzilli, C. Rucci, A. Vaiarelli,Cimadomo D, Ubaldi FM, Foresta C and Ferlin A., "Male factor infertility and assisted reproductive technologies: indications, minimum access criteria and outcomes," *Journal of Endocrinological Investigation* 46, no. 6 (2023): 1079-1085. doi: 10.1007/s40618-022-02000-4.
14. C. Douglas, N. Parekh, L. G. Kahn, R. Henkel, and A. Agarwal, "A novel approach to improving the reliability of manual semen analysis: a paradigm shift in the workup of infertile men," *The World Journal of Men's Health* 39, no. 2 (2021): 172-185.
15. C. Barbăroșie, A. Agarwal, and R. Henkel, "Diagnostic value of advanced semen analysis in evaluation of male infertility," *Andrologia* 53, no. 2 (2021): e13625.
16. M. K. Panner Selvam, R. F. Ambar, A. Agarwal, and R. Henkel, "Etiologies of sperm DNA damage and its impact on male infertility." *Andrologia* 53, no. 1 (2021): e13706.
17. J. A. Marinaro, and P. N. Schlegel, "Sperm DNA Damage and Its Relevance in Fertility Treatment: A Review of Recent Literature and Current Practice Guidelines," *International journal of molecular sciences* 24, no.2 (2023): 1446.
18. G. Gajski, S. Ravlić, R. Godschalk, A. Collins, M. Dusinska, and G. Brunborg, "Application of the comet assay for the evaluation of DNA damage in mature sperm," *Mutation Research/Reviews in Mutation Research* 788, (2021): 108398.
19. K. Liu, X. Mao, F. Pan, Y. Chen, and R. An, "Correlation analysis of sperm DNA fragmentation index with

semen parameters and the effect of sperm DFI on outcomes of ART," *Scientific reports* 13, no.1 (2023): 2717.

20. H. Debbarh, M. Jamil, H. Jelloul, A. Zakaria, N. Louanjli, and R. Cadi, "Assessment of cell-free DNA and apoptosis in an oocyte microenvironment: promising biomarkers to predict intracytoplasmic sperm injection outcomes." *Zygote* 31, no. 3 (2023): 296-302.
21. Zhang F, Li J, Liang Z, Wu J, Li L, Chen C, Jin F and Tian Y., "Sperm DNA fragmentation and male fertility: a retrospective study of 5114 men attending a reproductive center." *Journal of assisted reproduction and genetics* 38, (2021): 1133-1141. doi:10.1007/s10815-021-02120-5.
22. Ferrigno, A., Ruvolo, G., Capra, G., Serra, N., and Bosco, L. "Correlation between the DNA fragmentation index (DFI) and sperm morphology of infertile patients," *Journal of assisted reproduction and genetics* 38, (2021): 979-986.
23. K. Liu, X. Mao, F. Pan, Y. Chen, and R. An, "Correlation analysis of sperm DNA fragmentation index with semen parameters and the effect of sperm DFI on outcomes of ART," *Scientific reports* 13, no. 1 (2023): 2717.
24. Zhang K, Xu XH, Wu J, Wang N, Li G, Hao GM and Cao JF., "Decreased AKAP4/PKA signaling pathway in high DFI sperm affects sperm capacitation," *Asian Journal of Andrology* 26, no. 1 (2024): 25-33. doi: 10.4103/aja202329.
25. L. Farahani, T. Tharakan, T. Yap, J. W. Ramsay, C. N. Jayasena, and S. Minhas, "The semen microbiome and its impact on sperm function and male fertility: A systematic review and meta-analysis," *Andrology* 9, no. 1 (2021): 115-144.
26. Cannarella R, Condorelli RA, Gusmano C, Garofalo V, Aversa A, Calogero AE and La Vignera S., "Predictive role of 17α-hydroxy-progesterone serum levels of response to follicle-stimulating hormone in patients with abnormal sperm parameters," *Fertility and Sterility* 120, no. 6 (2023): 1193-1202.doi: 10.1016/j.fertnstert.2023.09.013.
27. Liu P, Shi J, Sheng D, Lu W, Guo J, Gao L, Wang X, Wu S, Feng Y, Dong D, Huang X and Tang H., "Mitopherogenesis, a form of mitochondria-specific ectocytosis, regulates sperm mitochondrial quantity and fertility," *Nature cell biology* 5, no. 11 (2023): 1625-1636.
28. Zhou L, Liu H, Liu S, Yang X, Dong Y, Pan Y, Xiao Z, Zheng B, Sun Y, Huang P, Zhang X, Hu J, Sun R, Feng S, Zhu Y, Liu M, Gui M and Wu J., "Structures of sperm flagellar doublet microtubules expand the genetic spectrum of male infertility," *Cell* 186, no. 13 (2023): 2897-2910.e19.doi: 10.1016/j.cell.2023.05.009.
29. G. Chen, and B. Zheng, "Effect of macrophages in semen on sperm quality," *Reproductive Biology and Endocrinology* 19, (2021): 38. doi:10.1186/s12958-021-00724-1.
30. Ten J, Guerrero J, Linares Á, Rodríguez-Arnedo A, Morales R, Lledó B, Llácer J and Bernabeu R., "Sperm DNA fragmentation on the day of fertilisation is not associated with assisted reproductive technique outcome independently of gamete quality," *Human Fertility* 25, no. 4 (2022): 706-715. doi: 10.1080/14647273.2021.1877364.
31. Zhang H, Zhu FY, He XJ, Tang SH, Long T, Peng L, Zhang HM, Zou ZZ, Xiong Z and Zhang XP., "The influence and mechanistic action of sperm DNA fragmentation index on the outcomes of assisted reproduction technology," *Open Life Sciences* 18, no. 1 (2023): 20220597.