

Impact of Paternal Age on DNA Fragmentation Index and Its Consequences for Embryo Development Following Intracytoplasmic Sperm Injection

Ria Margiana¹, Maitra D Wen², Silvia W Lestari³, Gito R Wasian⁴

Received on: 18 September 2025; Accepted on: 30 January 2026; Published on: 20 March 2026

ABSTRACT

Paternal age and sperm DNA fragmentation index (DFI) have become significant determinants of male infertility and assisted reproductive technology (ART) success rate. This article aims to establish the relationship between increased DFI and advanced paternal age and the developmental potential of embryos where intracytoplasmic sperm injection (ICSI) has been used. Sperm DNA integrity was measured on 150 participants aged 25–55 using the sperm chromatin dispersion (SCD) assay; DFI levels were further classified into subclinical, transition, and clinical abnormality ranges. Analysis of the findings showed a positive relationship between the age of the father and DFI ($r = 0.475, p < 0.001$), and older men had notably higher scores of DFI. In turn, DFI at the alternative level was negatively correlated with fertilization rates ($r = -0.416, p < 0.001$) and the quality of embryos on days 1 and 3 after ICSI. The results outlined the role of sperm DNA fragmentation when it comes to the success rate of ART, especially for patients of advanced age. Clinical relevance focuses on DFI testing and individual treatments that improve sperm selection [intracytoplasmic morphologically selected sperm injection (IMSI), physiological intracytoplasmic sperm injection (PICS)] and antioxidants due to age-associated oxidative damage. In this way, the study solves the research gap on the connection between paternal age, DFI, and ART success, which can help improve reproductive satisfaction and patient education on potential risks associated with delayed fatherhood. Subsequent studies must confirm the results and investigate new approaches to enhance ART outcomes in males with high DFI.

Keywords: DNA fragmentation index, Embryo development, Intracytoplasmic sperm injection, Paternal age, Reproductive health.

Journal of South Asian Federation of Obstetrics and Gynaecology (2026): 10.5005/jp-journals-10006-2856

INTRODUCTION

Infertility is a problem that affects almost one in eight couples around the world. Half of infertility cases are due to male infertility, and sperm quality is a significant determinant of successful conception.¹ While other forms of male infertility management have declined as the primary options in dealing with male infertility, intracytoplasmic sperm injection (ICSI) has enabled couples with male factor infertility to conceive.² However, despite advances in assisted reproductive technology (ART), success rates remain uncertain, and many factors affect outcomes. More and more, this is being recognized as an essential factor in male fertility, with research suggesting that sperm quality and fertilization capacity decrease as men age. One of the significant biomarkers of sperm quality is the DNA fragmentation index (DFI), which measures the extent of sperm DNA damage. High levels of DFI are associated with reduced fertility, decreased sperm motility, and decreased embryo development.³ This study focuses on determining the role of paternal age in affecting DFI and ICSI outcomes. Therefore, we hypothesize that advanced paternal age is associated with increased sperm DNA fragmentation, negatively impacting fertilization rates and embryonic quality in ICSI.

Introduction to Paternal Age and Male Infertility

The association of advanced paternal age with male infertility and the use of ART, especially ICSI, has instead become a key interest in current reproductive health care. Several research studies have revealed an increase in paternal age resulting from aspects such as delayed age of marriage and career advancement worldwide, which has increased the need for ART solutions.⁴ Nearly half of infertility cases are caused by male infertility, especially low sperm quality,

¹Department of Anatomy, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

²Department of Andrology, Faculty of Medicine, Universitas Airlangga Klinik Bocah Indonesia, Jakarta, Indonesia

^{3,4}Department of Biology, Faculty of Medicine, Klinik Bocah Indonesia, Jakarta, Indonesia

Corresponding Author: Ria Margiana, Department of Anatomy, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, Phone: +628111775515, e-mail: ria.margiana11@gmail.com

How to cite this article: Margiana R, Wen MD, Lestari SW, *et al.* Impact of Paternal Age on DNA Fragmentation Index and Its Consequences for Embryo Development Following Intracytoplasmic Sperm Injection. *J South Asian Feder Obst Gynae* 2026;18(1):84–90.

Source of support: Nil

Conflict of interest: None

which makes it critically important for the development of such ART as ICSI.⁵ Nevertheless, doubts remain high as a consequence of ICSI for male infertility, eliminating natural sperm selection and propensity to transfer genetic disorders.⁶

As well indicated in previous studies, the increase in paternal age is associated with poor quality sperms, less motility, and abnormal morphology, topped by reduced DNA integrity, which tends to have negative impacts on ART.⁷ DNA fragmentation index, a factor directly related to age, may alter the subsequent embryo quality and the implantation rate.⁸ This review discusses the relationship between paternal age, DFI, and the capacity to predict embryo development in the context of ICSI to help practitioners and offer insight into further study.

DNA Fragmentation Index

Sperm DFI is a sensitive and accurate marker of sperm quality that can explain sperm DNA and its fragility.³ The possible causes of sperm DNA fragmentation include oxidative stress, impaired DNA repair mechanisms during spermatogenesis, and apoptosis.^{9,10} Sperm DFI is assessed through tests like the sperm chromatin dispersion (SCD) test and sperm chromatin structure assay (SCSA) to know the degree to which sperm DNA quality impacts fertility in men.

Higher DFI has also been linked with poor sperm motility, morphology, and fertilization, which influences embryo development and clinical ICSI pregnancy rates.¹¹ Similarly, Esteves et al. identified oxidative stress as an essential factor that directly causes DNA damage and subsequent chromatin alteration in sperms, as well as poor outcomes of ART.¹² Liu et al. similarly found low DFI values with sperm survival rates and motility velocity, also endorsing the use of DFI in the diagnosis.¹³

Impact of Advanced Paternal Age on DFI

Studies prove that higher paternal age raises the levels of sperm DFI, one of the prominent markers of male infertility. Sharma et al. noted that increased DFI is caused by advanced paternal age due to decreased DNA repair mechanisms and increased oxidative stress.⁷ Their study stressed that DNA fragmentation increases dramatically in men above 40 years, indicating lower sperm quality and unfavorable fertility results.

Oxidative stress has been highlighted as the central determinant of age-related sperm DNA damage. Nguyen-Powanda and Robaire confirmed that the antioxidant defense capacity declines with age, whereas the level of ROS increases and contributes to DNA damage in sperms.¹⁴ This study also established that increased oxidative stress is associated with reduced sperm chromatin functionality and increased apoptosis. Likewise, Bibov et al. highlighted that randomized oxidative stress is another primary factor leading to DFI increases in aging males, affecting spermatogenesis.¹⁵

Moskovtsev et al. directly measured this trend, doubling DFI from 15.2% in men younger than 30–32% in men older than 45.¹⁶ Sharma et al. supported these findings, observing that DFI has significantly enhanced for 36- to 57-year-old male participants compared to young male participants.⁷ In addition, Kaltsas et al. determined that increased levels of DFI hurt the level and implantation rate of embryos in ART.⁴ Taken together, the above works highlight the importance of counseling the male aging population on the reproductive effects of an elevated DFI, with a special focus on practical strategies for managing oxidative damage to sperm.

Consequences of Elevated DFI for ART Outcomes

High DFI hurts ART outcomes. Bibov et al. observed that any level of DFI $\geq 30\%$ dramatically diminishes the quality of embryos at day 3 and blastocyst development rates and elevates miscarriage rates in cases with no male factor infertility.¹⁵ Along the same line, Wang et al. realized that relying on cycles with high DFI yielded a low number of good quality embryos and live birth rates, supporting the role of DFI in estimating the prognosis of ART.¹⁷ In ICSI, Zhu et al. observed that low and high DFI have adverse impacts on delivering fresh or frozen embryos but have varying effects on fertilization and miscarriage rates.¹⁸ In line with this, Li et al. described higher miscarriage rates and reduced birth weight in infant subjects by DFI $>30\%$, indicating dangers to offspring health.¹⁹

Some of the measures used to prevent or lessen the extent of DFI are improved methods of sperm selection. Esteves et al. described Testi-ICSI as highly effective with better live birth outcomes, where DFI was found to be higher in testicular sperm than in ejaculate sperm.¹² Some methods, such as intracytoplasmic morphologically selected sperm injection (IMSI) and physiological intracytoplasmic sperm injection (PICS), are also used to distance superior-quality sperm with intact DNA.^{20,21} However, their efficiency is still in a state of controversy. Moreover, many techniques, like the ejaculation issue and density gradient centrifugation, can successfully lower DFI levels.²² Altogether, these data stress the importance of personalized strategy in ART for those patients with elevated DFI, using enhanced diagnostic and specific sperm selection, leading to better fertilization, embryo quality, and outcomes.

METHODS

Study Design

This study conducted a retrospective cohort analysis for 2024 in two fertility centers in Jakarta and Tangerang City, Indonesia. The ethical clearance number for the study was KET-405/UN2.F1/ETIK/PPM.00.02/2025 with protocol number 25-01-0119 from Universitas Indonesia. This study involved 150 male patients with male factor infertility who were undergoing the treatment of ICSI. All male patients enrolled in this study were ICSI candidates aged 25–55 years and diagnosed with infertility factors such as oligozoospermia, asthenozoospermia, or teratozoospermia. Male partners provided sperm samples through masturbation by avoiding sexual contact for 2–7 days prior to sample provision. According to the proposed criteria, persons who met these conditions were considered for inclusion in the study. Nevertheless, subjects with azoospermia or severe oligospermia of sperm concentration <1 million sperm/mL were excluded from this study. Moreover, cases with surgically retrieved sperm or frozen sperm cycles were not included so that comparability in evaluating sperm quality could be maintained. Any participant with incomplete records or data was excluded to avoid skewing the study findings.

The age of the father was determined at the time the couple was undergoing the ICSI treatment process. This variable was then used to form patient groups and compare their results according to age. Then, the SCD test was performed to determine the extent of sperm DNA fragmentation, which was considered poor. These assessments defined the DFI or DFI to clarify how fragmented DNA is. The development of embryos by ICSI was described on the first and third days after fertilization. The embryos were classified into three morphological classes based on these parameters: Good, medium, and poorly developed. These categories helped set up a trend of creating a link between the age of the father and the DFI to embryonic outcomes. Such an approach enabled the study to compare the success of ICSI to the other related aspects of fatherhood, such as the density of the sperm DNA and age, to establish a correlation between the two to increase the success of ICSI.

The fertilization rate (%) denotes the percentage of oocytes (eggs) that successfully achieve fertilization upon exposure to sperm. This parameter is essential for evaluating the efficacy of ART. The fertilization rate is determined by dividing the quantity of fertilized oocytes by the total number of mature oocytes and subsequently multiplying by 100%. A high fertilization rate signifies superior sperm quality and ideal laboratory circumstances, whereas

a low rate may indicate problems with sperm functionality, oocyte quality, or culture conditions.

D1 embryo quality (%) quantifies the percentage of embryos exhibiting normal fertilization and early cleavage on the first day post-fertilization. This is assessed by the presence of two pronuclei (2PN), indicating proper fertilization. Embryos exhibiting an aberrant pronuclei count (e.g., 1PN or 3PN) may possess chromosomal anomalies and are generally removed from subsequent development or transfer.

D3 good quality (%) denotes the proportion of embryos achieving an optimal cleavage stage with perfect morphology on the third day following fertilization. High-quality embryos demonstrate appropriate cellular division, little fragmentation, and symmetrical blastomeres. These embryos possess the greatest chance for successful implantation and gestation.

D3 intermediate quality (%) denotes embryos exhibiting moderate traits for cell count, fragmentation, and symmetry on day 3. These embryos may possess modest abnormalities yet remain viable for embryo transfer or cryopreservation. Although their implantation potential is inferior to that of high-quality embryos, they can still lead to healthy pregnancies.

D3 poor quality (%) refers to embryos exhibiting substantial fragmentation, sluggish or irregular cleavage, or atypical blastomere shape on day 3. These embryos exhibit a decreased probability of implantation and successful gestation owing to compromised development. In certain instances, they may continue to be utilized in treatment cycles; nevertheless, their clinical outcomes are typically inferior to those of higher-quality embryos.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software, version 20. Before comparison, the Kolmogorov–Smirnov test was first performed for normality. Then the Kruskal–Wallis test was used. Spearman’s rank correlation coefficient was used to compare the relationship between paternal age and DFI.^{23,24} The effect of paternal age and DFI on fertilization rates and embryo quality was analyzed using linear regression equations. Statistical significance was accepted when the p -value was < 0.05 .

RESULTS

One hundred fifty male patients participated in the study. The paternal age of participants varied from 25 to 55 years, with a mean $\pm$ SD of 37.05 ± 6.23 years. The DFI levels varied between 10 and 55%, with elevated DFI noted in older males. Paternal age was classified into three unique categories: The subclinical group (25–35 years), the transition group (ages 35–45), and the clinical group (ages above 45). The subclinical group demonstrated the lowest mean DFI levels, averaging 15% (with a range of 10% to 25%), signifying better sperm DNA. In the transition group, moderate DFI levels were observed, averaging 30% (25–40%). The clinical group exhibited the highest DFI levels, averaging 40% (35–55%). The age distribution in Figure 1 exhibited a minor bias towards older demographics, with a greater percentage of participants in the 35–45 age bracket.

As can be seen in Figure 2, the levels of DFI showed a pattern of gradual increase with increasing age. Individuals in the subclinical group (25–35 years) typically exhibited lower DFI values (mean ~15%, range 10–25%), whereas those in the transition group (35–45 years) demonstrated moderate levels (mean ~30%, range 25–40%).

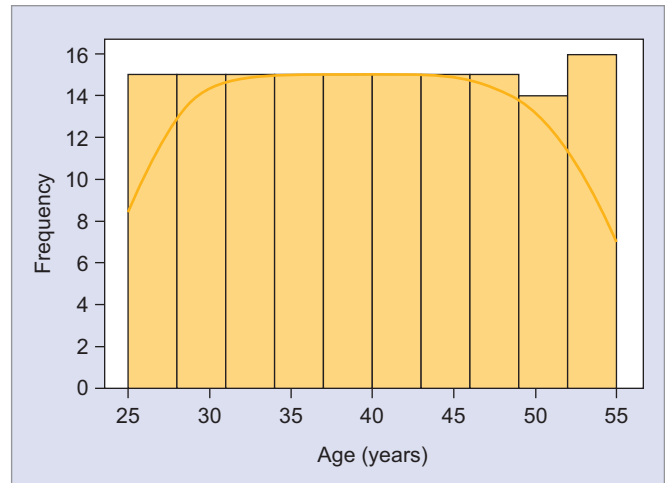


Fig. 1: Age distribution of subjects

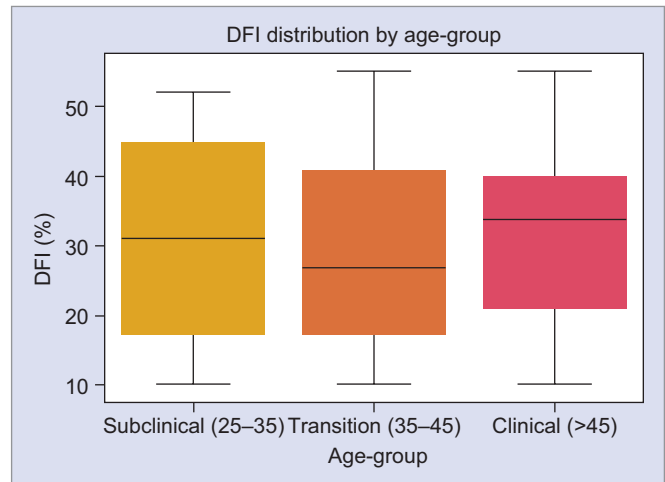


Fig. 2: DNA fragmentation index distribution categorized by age-group

The clinical group (>45 years) exhibited the greatest DFI values (mean ~40%, range 35–55%), with increased variability noted within this age category. This indicates that the integrity of sperm DNA deteriorates with age, potentially affecting reproductive results (Fig. 2).

As can be seen in Figure 3, this figure provides a clearer knowledge of the correlations between important semen parameters, embryo quality, and DFI. All of these relationships were determined using a series of correlation analyses. For the purpose of visualizing these associations, scatter plots with trend lines were utilized, and statistical correlations were computed in order to evaluate the importance of these relationships (Fig. 3).

Age vs DFI

A weak positive correlation was observed ($r = 0.475$, $p = < 0.001$), suggesting that DFI tends to increase slightly with age. However, the correlation is statistically significant, indicating that age alone does not strongly predict sperm DNA fragmentation in this dataset. This implies that other factors, such as lifestyle, genetic predisposition, and environmental exposures, may contribute more significantly to DFI levels.

Correlation analysis

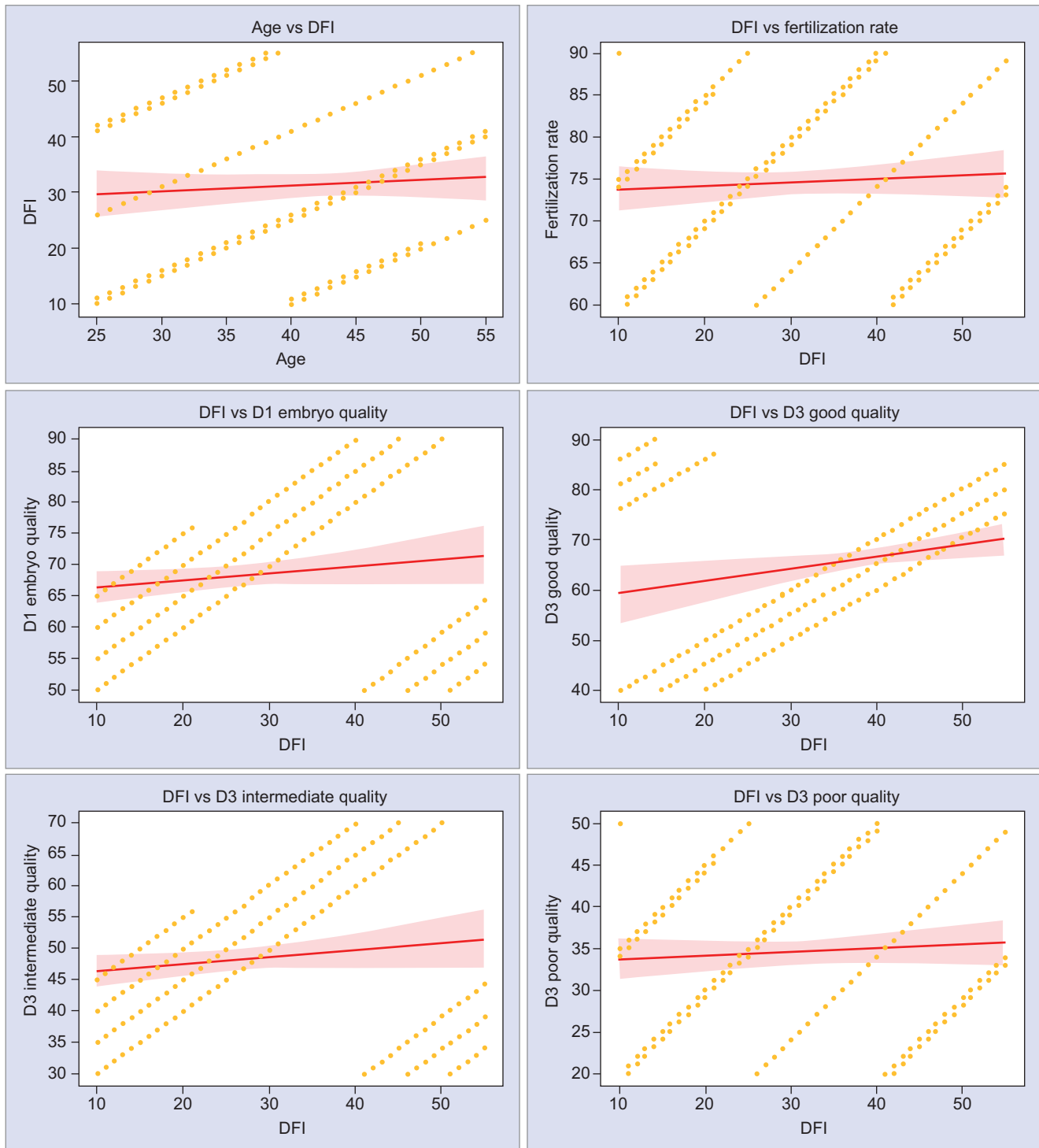


Fig. 3: Higher percentages of DFI are associated with lower sperm motility percentages

DFI vs Fertilization Rate

A negative correlation ($r = -0.416, p < 0.001$) was found, indicating that higher DFI levels may affect the oocyte, and the effect is statistically significant. This suggests that despite increased sperm DNA damage, fertilization can still occur, likely due to compensatory mechanisms in the oocyte that can repair damaged sperm DNA.

DFI vs Day 1 (D1) Embryo Quality

A weak negative correlation ($r = -0.391, p < 0.005$) was observed between DFI and embryo quality on day 1. This suggests that while higher DFI may have a mild impact on early embryo development, the effect is strong enough to be considered a major determinant of embryo quality at this stage.

Table 1: Correlation between paternal age, DFI, and ICSI outcomes

Parameter	Correlation (r)	p-value
Paternal age and DFI	+0.475	<0.001
DFI and fertilization rate	-0.416	<0.001
DFI and D1 embryo quality	-0.391	<0.005
DFI and D3 embryo quality (good)	-0.232	<0.005
DFI and D3 embryo quality (intermediate)	-0.257	<0.005
DFI and D3 embryo quality (poor)	-0.287	<0.005

DFI vs Day 3 (D3) Good Embryo Quality

A moderate negative correlation ($r = -0.232$, $p = <0.005$) was identified, indicating that higher DFI levels are significantly associated with a lower percentage of good quality embryos on day 3. This suggests that sperm DNA fragmentation plays a more critical role in later stages of embryo development, likely due to the activation of the paternal genome, which starts influencing embryonic growth after fertilization.

DFI vs Day 3 (D3) Intermediate Embryo Quality

A weak negative correlation ($r = -0.257$, $p = <0.005$) was found, meaning that DFI significantly influence the proportion of embryos classified as intermediate quality. This suggests that while some embryos with moderate DFI may show some degree of developmental delay, the overall impact is substantial.

DFI vs Day 3 (D3) Poor Embryo Quality

A weak negative correlation ($r = -0.287$, $p = <0.005$) was observed, indicating that DFI levels may contribute to poor-quality embryos; the effect is statistically significant in this dataset. This suggests that, despite of sperm DNA fragmentation, oocyte quality and embryo culture conditions may play a larger role in determining poor embryo outcomes.

Key Findings

- DNA fragmentation index has a statistically significant negative correlation with day 3 good embryo quality ($p = 0.006$), meaning that higher DNA fragmentation is linked to a lower proportion of good quality embryos on day 3.
- Age strongly predict DFI ($p = <0.001$), suggesting that sperm DNA fragmentation is influenced by paternal age.
- Correlations were found between DFI and fertilization rate or other embryo quality metrics, indicating that sperm DNA integrity plays a role, but other embryological and maternal factors may influence embryo development and implantation success.

Table 1 indicates a substantial positive association between paternal age and the DFI, with a correlation coefficient of $r = 0.475$ and $p < 0.001$. This suggests that with advancing paternal age, there is a significant increase in sperm DNA fragmentation, indicating a deterioration of sperm DNA integrity with time.

Elevated DFI levels adversely affected ICSI outcomes. A notable negative connection was seen between DFI and fertilization rates ($r = -0.416$, $p < 0.001$), indicating diminished fertilization success with heightened DNA fragmentation. Moreover, embryo quality on both day 1 (D1) and day 3 (D3) exhibited substantial negative relationships with DFI. The correlation for D1 embryo quality was $r = -0.391$ ($p < 0.005$), but D3 embryo quality exhibited negative

correlations across good ($r = -0.232$), intermediate ($r = -0.257$), and poor ($r = -0.287$) categories, all with p -value < 0.005 .

DISCUSSION

The findings of this study confirm a significant positive correlation between paternal age and the DFI, corroborating previous research emphasizing the decline in sperm quality with advancing paternal age. Specifically, older men exhibited higher DFI levels, with the clinical group (ages above 45) showing the highest DNA fragmentation. This aligns with Sharma et al. and Nguyen-Powanda and Robaire, who attributed such increases to diminished DNA repair mechanisms and heightened oxidative stress in aging spermatozoa.^{7,14} These biological processes not only undermine sperm chromatin integrity but also elevate risks to embryonic viability.

Figure 2 shows that increased DFI was observed in fertilization rates and embryo quality. The study resonates with Bibov et al. and Sharma et al., who pointed towards reduced embryo development as well as high miscarriage rates brought about by high DFI.^{7,15} This decline in embryo quality on days 1 and 3 indicates that sperm DNA integrity is paramount in embryonic development. These results suggest that DFI is a valid biomarker to gauge the chances of ART success within older male populations.

From the mechanical point of view, it is evident that oxidative stress has a causal connection with sperm DNA damage. Other authors like Nguyen-Powanda and Robaire illustrated that aging weakens the antioxidant protection system, hence generating an increased amount of ROS that, in turn, increases DNA damage.¹⁴ This concurs with the current study, where DFI was higher in the participants of higher age. Additionally, the reduction in DNA repair capacity in aging sperm cells, as highlighted by Bibov et al., creates the rationale for these trends observed in statistics.¹⁵ Wang et al. also found that cycles with high DFI yielded a low number of good quality embryos and live birth rates, supporting the role of DFI in estimating the prognosis of ART.¹⁷

Clinical Implications

Since father age and DFI have such a strong relationship with ICSI outcomes, clinicians must give heed to paternal age and sperm DNA fragmentation in counseling couples for ART.²⁴ Male subjects in the clinical age-group more significant than 45 years should be informed of risks associated with advanced paternal age, such as increased DFI and lower efficacy of ART. The male subjects have to be informed about miscarriage rates and low birth weight. Zhu et al. found that low and high DFI have adverse impacts on delivering fresh or frozen embryos, but give varying effects on fertilization and miscarriage rates. Li et al. described higher miscarriage rates and lower birth weight in infant subjects by DFI >30%.¹⁹

There is an advantage in doing routine tests for sperm DNA fragmentation since it may provide relevant information on sperm quality as well as help in the appropriate management of male fertility treatment.²⁵ Sperm selection techniques, such as IMSI, for older male clients may improve ART outcomes in older men.

Limitations of the Study

Although this study offers a valuable contribution to determining the nature of the correlation between paternal age, DFI, and ICSI results, many limitations need to be acknowledged to understand the results adequately. While 150 participants are sufficient to provide statistically meaningful results, the scope of generalization

of the findings is limited. The extent to which respondents have a diverse geographical background and a better socioeconomic background would make the study more valid and applicable.²⁶ Other factors that could significantly predict DFI and fertility—lifestyle and environmental factors, including smoking, alcohol consumption, obesity, and endocrine disruptors—were not standardized or studied in this research. Using these variables in subsequent studies might enhance understanding of the factors relating to DFI and reproductive success.

A possible weakness of the study is that the data used are derived from only two fertility facilities. Inaccuracies in repeated experiments in different venues may arise from differences in approach, personnel expertise, and environmental parameters across laboratories and affiliated clinics.²⁷ Therefore, the retrospective format of the study limits its ability to show causation to a great extent. Even though significant relations were demonstrated, longitudinal studies are required to confirm the causal relationships between paternal age, DFI, and ICSI results.

Also, this study did not consider the effects of several new techniques in sperm selection, including IMSI or PICSI. These approaches may help to reduce the impact of high DFI levels on ICSI outcomes and suggest potential ways for improving the treatment outcomes in males with high DFI levels. Subsequent research to overcome these limitations can provide a more comprehensive and clinically relevant analysis of paternal factors about ICSI outcomes.

Novelty of the Study

This study provides several new findings in reproductive medicine focusing on ART and male causes of infertility. This current research is the first study in Indonesia exploring the link between increasing paternal age and the DFI. This research fills the gap in understanding the extent and nature of male fertility. It presents findings relevant to the Indonesian population due to the unconditional adherence to a region's sociodemographic and healthcare systems. These are critical for the fine-tuning of fertility treatments and counseling around the country. Secondly, the study used a distinctive method for age stratification, categorizing paternal age into three specific groups: Preclinical (25–35 years), early manifestation (35–45 years), and full manifestation (>45 years). This stratification helps to differentiate further how age affects sperm DNA damage and its consequent effect on ICSI. The study identifies the age-specific risks and impacts in the timetable of male reproductive health more accurately.

This research adopts a comprehensive analytical approach by relating DFI levels to all the ICSI outcome measures, such as fertilization rates and quality of embryos on D1 and D3. These findings provide a strong foundation to examine the complex association between paternal age, sperm DNA quality, and ART effectiveness. Therefore, the results provide clear clinical recommendations indicating the concrete values of paternal age and DFI. These standards enable the doctor to provide the patient with quicker information concerning their reproductive features and the possibility of conception with the help of artificial methods. This may be useful in clinical practice and may even foster better results for the couples being treated for reproductive problems.

CONCLUSION

This study found that the father's age is positively related to DFI, showing that advanced paternal age leads to high sperm DNA fragmentation. Increased paternal age >45 has been associated

with high DFI levels, poor ICSI fertilization rates, and embryo quality. Therefore, paternal age should be included in fertility assessment along with DFI, which will eventually contribute to improving ART efficiency and the couple's guidance. Further research has to be done to establish the potential strategies that might be useful in reducing the impact of sperm DNA fragmentation in aging males.

Sources of Funding

This work was funded by PUTI Scheme Q1 (Program Unggulan Terpadu Indonesia) from Universitas Indonesia in 2025 with contract number PKS-104/UN2.RST/HKP.05.00/2025.

Data Availability

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

ACKNOWLEDGMENT

Nil.

AUTHOR'S CONTRIBUTIONS

Conceptualization: RM Data curation: RM Formal analysis: RM Funding acquisition: RM. Investigation: RM Methodology: RM Project administration: RM. Resources: RM. Software: RM. Supervision: RM. Validation: RM. Visualization: RM. Writing – Original draft: RM. Writing – review and editing: RM.

REFERENCES

1. Leathersich S, Hart RJ. Immune infertility in men. *Fertil Steril* 2022;117(6):P1121–P1131. DOI: 10.1016/j.fertnstert.2022.02.010.
2. Xie P, Cheung S, Kocur O, et al. Intracytoplasmic sperm injection is still the best management of male factor infertility. *Fertil Steril* 2024;121(4):563–575. DOI: 10.1016/j.fertnstert.2024.02.025.
3. Stavros S, Potiris A, Molopodi E, et al. Sperm DNA fragmentation: Unraveling its imperative impact on male infertility based on recent evidence. *Int J Mol Sci* 2024;25(18):10167. DOI: 10.3390/ijms251810167.
4. Kaltsas A, Zikopoulos A, Vrachnis D, et al. Advanced paternal age in focus: Unraveling its influence on assisted reproductive technology outcomes. *J Clin Med* 2024;13(10):2731. DOI: 10.3390/jcm13102731.
5. Sengupta P, Dutta S, Jegasothy R, et al. "Intracytoplasmic sperm injection (ICSI) paradox" and "andrological ignorance": AI in the era of fourth industrial revolution to navigate the blind spots. *Reprod Biol Endocrinol* 2024 13;22(1). DOI: 10.1186/s12958-024-01193-y.
6. Borges E Jr, Zanetti BF, Braga DP de AF, et al. Overcoming male factor infertility with intracytoplasmic sperm injection. *Rev Assoc Med Bras* 2017;63(8):697–703. DOI: 10.1590/1806-9282.63.08.697.
7. Sharma R, Agarwal A, Rohra VK, et al. Effects of increased paternal age on sperm quality, reproductive outcome and associated epigenetic risks to offspring. *Reprod Biol Endocrinol* 2015;13:35. DOI: 10.1186/s12958-015-0028-x.
8. Halvaei I, Litzky J, Esfandiari N. Advanced paternal age: Effects on sperm parameters, assisted reproduction outcomes and offspring health. *Reprod Biol Endocrinol* 2020;18(1):110. DOI: 10.1186/s12958-020-00668-y.
9. Muratori M, Marchiani S, Tamburrino L, et al. Sperm DNA fragmentation: Mechanisms of origin. *Adv Exp Med Biol* 2019;1166:75–85. DOI: 10.1007/978-3-030-21664-1_5.
10. Yang H, Li G, Jin H, et al. The effect of sperm DNA fragmentation index on assisted reproductive technology outcomes and its relationship with semen parameters and lifestyle. *Transl Androl Urol* 2019;8(4):356–365. DOI: 10.21037/tau.2019.06.22.

11. Sivanarayana T, Krishna ChR, Prakash GJ, et al. Sperm DNA fragmentation assay by sperm chromatin dispersion (SCD): Correlation between DNA fragmentation and outcome of intracytoplasmic sperm injection. *Reprod Med Biol* 2013;13(2):87–94. DOI: 10.1007/s12522-013-0168-7.
12. Esteves SC, Zini A, Coward RM, et al. Sperm DNA fragmentation testing: Summary evidence and clinical practice recommendations. *Andrologia* 2021;53(2):e13874. DOI: 10.1111/and.13874.
13. Liu KS, Mao XD, Pan F, et al. Correlation analysis of sperm DNA fragmentation index with semen parameters and the effect of sperm DFI on outcomes of ART. *Sci Rep* 2023;13(1):2717. DOI: 10.1038/s41598-023-28765-z.
14. Nguyen-Powanda P, Robaire B. Oxidative stress and reproductive function in the aging male. *Biology (Basel)* 2020;9(9):282. DOI: 10.3390/biology9090282.
15. Bibov MY, Kuzmin AV, Alexandrova AA, et al. Role of the reactive oxygen species induced DNA damage in human spermatozoa dysfunction. *AME Med J* 2018;3:19. DOI: 10.21037/amj.2018.01.06.
16. Moskovtsev SI, Willis J, Mullen JBM. Age-related decline in sperm deoxyribonucleic acid integrity in patients evaluated for male infertility. *Fertil Steril* 2006;85(2):496–499. DOI: 10.1016/j.fertnstert.2005.05.075.
17. Wang Q, Gu X, Chen Y, et al. The effect of sperm DNA fragmentation on in vitro fertilization outcomes of unexplained infertility. *Clinics (Sao Paulo)* 2023;78:100261. DOI: 10.1016/j.clinsp.2023.100261.
18. Zhu CH, Chen F, Zhang S, et al. Influence of sperm DNA fragmentation on the clinical outcome of in vitro fertilization-embryo transfer (IVF-ET). *Front Endocrinol (Lausanne)* 2022;13:945242. DOI: 10.3389/fendo.2022.945242.
19. Li F, Duan X, Li M, et al. Sperm DNA fragmentation index affect pregnancy outcomes and offspring safety in assisted reproductive technology. *Sci Rep* 2024;14(1):356. DOI: 10.1038/s41598-023-45091-6.
20. Oseguera-López I, Ruiz-Díaz S, Ramos-Ibeas P, et al. Novel techniques of sperm selection for improving IVF and ICSI outcomes. *Front Cell Dev Biol* 2019;7:298. DOI: 10.3389/fcell.2019.00298.
21. Baldini D, Ferri D, Baldini GM, et al. Sperm selection for ICSI: Do we have a winner? *Cells* 2021;10(12):3566. DOI: 10.3390/cells10123566.
22. Umair M, Henning H, Stout TAE, et al. A modified flotation density gradient centrifugation technique improves the semen quality of stallions with a high DNA fragmentation index. *Animals* 2021;11(7):1973. DOI: 10.3390/ani11071973.
23. Ferrigno A, Ruvolo G, Capra G, et al. Correlation between the DNA fragmentation index (DFI) and sperm morphology of infertile patients. *J Assist Reprod Genet* 2021;38(4):979–986. DOI: 10.1007/s10815-021-02080-w.
24. Antonouli S, Papatheodorou A, Panagiotidis Y, et al. The impact of sperm DNA fragmentation on ICSI outcome in cases of donated oocytes. *Arch Gynecol Obstet* 2019;300(1):207–215. DOI: 10.1007/s00404-019-05133-9.
25. Durairajanayagam D. Commentary: The value of testing sperm DNA fragmentation in infertile men. *Transl Androl Urol* 2017;6(Suppl 4):S678–S680. DOI: 10.21037/tau.2017.03.42.
26. Khan JA, Raman AM, Sambamoorthy N, et al. *Research Methodology (Methods, Approaches and Techniques)*. SAN International Scientific Publications; 2023.
27. Wilkinson J, Stocking K. Study design flaws and statistical challenges in evaluating fertility treatments. *Reprod Fertil* 2021;2(2):C9–C21. DOI: 10.1530/RAF-21-0015.