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Study a Relationship Between Age, Body Mass Index, and Sperm Parameters with Sperm DNA Fragmentation Levels in Iraqi Infertile Patients

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Abstract

Background: Male infertility is complex and accounts for approximately 50% of couples facing conception challenges. Factors such as body mass index, age, and sperm parameters play significant roles. Recognizing their impact is vital for diagnosis and treatment.

Aim of the study: The study aimed to evaluate DNA integrity in individuals with unexplained infertility and assess its correlation with age, body mass index, and semen parameters.

Materials and methods: In this study, 111 males who met the inclusion criteria were recruited for our study. Selected participants were grouped according to their age, body mass index, and semen parameters, including (sperm count, percentage motility, and morphology) with Aniline Blue Staining and Sperm Chromatin Dispersion Staining used in the IVF center-Baghdad.

Results: Our analysis found no significant correlations between Aniline Blue Stain and several parameters, including male age ($p = 0.382$), body mass index ($p = 0.238$), sperm concentration ($p = 0.117$), sperm morphology ($p = 0.229$), and sperm motility ($p = 0.079$). Thus, within our study, Aniline Blue Stain showed no statistically significant relationships with these factors. In contrast, Sperm Chromatin Dispersion Staining exhibited significant associations with sperm concentration ($p = 0.025$) and sperm morphology ($p = 0.039$) in our study population. However, male age ($p = 0.104$), body mass index ($p = 0.102$), and sperm motility ($p = 0.173$) did not demonstrate significant correlations with Halo Sperm Staining.

Conclusion: Evaluating sperm DNA damage through semen analysis is vital in male fertility screening, particularly for unexplained infertility for sperm parameters (sperm concentration and sperm morphology).

Keywords: Aniline blue staining, Body mass index, Male age, Semen parameters, Sperm chromatin dispersion staining

Introduction

Male infertility is a complex and multi-factorial condition characterized by the inability of a man to father a child in situations where pregnancy is achievable through normal sexual activity (Yu et al., 2022). Up to 15% of couples trying to conceive now experience infertility, and about 50 million couples worldwide (Kumar & Singh, 2015a). It's crucial to underline the following points: Up to 20–70% of infertility cases are caused by male

factors (coexisting with female factors), and only male factors account for 30% of these cases (Zegers-Hochschild et al., 2009). In addition, semen quality has declined globally over the past few decades, and paternal age is rising as more men choose to have children later in life (Patel et al., 2016).

Decreased sperm quality is the most important among the many causes causing male infertility (Rosiak-Gill et al., 2019). To assess reproductive potential, the standard parameters of semen analysis are insufficient (Stone et al., 2013). The ejaculate

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volume, sperm count, motility, vitality, and sperm morphology all show signs of semen quality degradation (Plastira et al., 2007). It should be mentioned that the sperm chromatin state also shows signs of aging's harmful effects. Adverse changes in every semen characteristic, however, are not always noticed simultaneously (Du Plessis et al., 2010).

It has been hypothesized that obesity may affect male fertility by lowering the quality of the sperm produced and the amounts of testosterone in the body (Chavarro et al., 2010a). Another thing that may be involved in obese people is the integrity of their sperm DNA, which may be caused by more damage due to oxidative stress (Hammiche et al., 2011). Many studies have shown that losing weight as part of a healthy lifestyle may improve erectile dysfunction and sperm parameters (Akingbemi, 2005). However, there is currently insufficient data to demonstrate causation or enhanced fertility after weight reduction management programs (Hammoud et al., 2006).

Advanced paternal age is indeed associated with several reproductive challenges, including increased DNA fragmentation in sperm, decreased sperm motility, and a higher likelihood of chromosomal abnormalities in offspring (Kaltsas et al., 2023). These factors collectively contribute to reduced fertility and an increased risk of genetic disorders in children born to older fathers (Chan & Robaire, 2022). As men grow older, their fertility can be affected, with both the quality and quantity of sperm generally diminishing over time (Skakkebaek et al., 2022). This natural age-related decrease in sperm quality and quantity can impact a man's ability to conceive with a partner (Seriki et al.).

While World Health Organization sperm parameters and DNA damage have traditionally been viewed as distinct aspects of male fertility assessment, recent research suggests they are more complementary than strongly linked (Cohen-Bacrie et al., 2009a). Understanding this relationship is crucial in comprehensively evaluating the paternal contribution to assisted reproductive technology failures (Pino et al., 2020a). Intriguingly, some findings studies reveal that sperm DNA fragmentation is not strongly correlated with conventional semen parameters. Consequently, it becomes imperative to incorporate a sperm DNA fragmentation assay as an additional step in the investigation of male fertility (Le et al., 2019). Other results underscore a significant disparity in sperm DNA damage between infertile and fertile males, particularly highlighting the higher levels of DNA damage in sperm with abnormal morphology and reduced motility

(Sheykh et al.). Notably, compromised seminal antioxidant status and elevated levels of lipid peroxidation and iron can adversely affect sperm nuclear integrity, leading to DNA breaks and potentially contributing to poor sperm morphology (Ammar et al., 2019).

It is becoming widely understood that diagnosing sperm DNA integrity with routine semen analysis is essential for guiding the treatment course in infertile couples. For the embryo's best possible growth and development, the sperm's DNA must be intact (Esposito et al., 2004).

In recent years, several tests have been developed to measure the structure of sperm chromatin, and these included direct and indirect methods; Aniline Blue Staining (ABS), the Terminal dUTPnick End Labeling (TUNEL), the Single Cell Gel Electrophoresis (COMET), the Acridine Orange (AO), the Chromomycin A3 (CMA3), the Sperm Chromatin Structure Assay (SCSA), and the Sperm Chromatin Dispersion (SCD) (Evenson, 2016; Tandara et al., 2013).

The rationale of this study is to address the existing knowledge gap and investigate the relationship between Sperm integrity and Male age, Body mass index, and Sperm parameters. Despite previous research in this area, there is still limited understanding of the relationship between them, so it is crucial to conduct a comprehensive examination of this relationship. So, this study aimed to elucidate whether there is a relationship between Sperm DNA fragmentation and men's age, weight mass, and sperm parameters including (sperm concentration, sperm morphology, and sperm motility).

Materials and methodology

We studied One hundred and eleven couples with unexplained infertility due to male factors between (November 2020–February 2021) at Al-Farah Specialist Fertility Center and IVF -Baghdad. Before undergoing Intra Uterine Insemination, all men with Normozoospermic: It is indicated that the semen sample has a Normal Sperm Count above 20 million sperm per milliliter, motility of at least 50% should show sperm progressive motility, and sperm morphology of at least 30% of the sperm cells should have normal morphology, which are essential factors for male fertility Table 1 (World Health Organisation, 1999). All unexplained infertile Males underwent an andrology evaluation of sperm DNA damage by sperm chromatin dispersion staining and aniline blue staining. Examinations were conducted on the married and included in this study were average men aged between <30 years and ≥40

Table 1. Reference values for semen analysis according to WHO 4th edition (World Health Organisation, 1999).

Parameter	Low references value in semen analysis
Volume	>2 ml
Sperm concentration	>20 million/ml
Sperm motility	>50% progressively motile (including Grade A + B)
Sperm morphology	>14% normal forms

years (Petersen et al., 2018), and who have a body mass index below 18.5 to above 40 according to WHO in Table 2 (Caballero, 2019). The study included weight in kilograms and height in centimeters for each person. In addition to the semen analysis parameters (sperm of concentration, movement, and shape) according to WHO 4th edition. For each sample, two analyzes were performed (aniline blue dye and sperm DNA damage) Table 3 (Evgeni et al., 2014). Samples were collected based on the patients' informed consent, consent, and ethical requirements of the Infertility Center, Ethics Committee No: March 2, 5157, August 10, 2020 AD).

Sperm chromatin dispersion test

The sperm chromatin dispersion (SCD) test, also known as the sperm Halo test, is a laboratory test used to evaluate the integrity of sperm DNA and

Table 2. Reference values for normal ^aBMI in humans according to WHO (Caballero, 2019). For adults over the age of 20, the BMI falls into one of the following categories.

Nutritional status	Body mass index
Underweight	below 18.5
Normal weight	18.5–24.9
Pre-obesity	25.0–29.9
Obesity class I	30.0–34.9
Obesity class II	35.0–39.9
Obesity class III	above 40

^a Body Mass Index by using a calculator the BMI Formula; Metric BMI Formula is BMI = (Weight in Kilograms/(Height in Meters × Height in Meters)).

Table 3. The Normal value in Sperm DNA Damage (Evgeni et al., 2014).

Sperm DNA fragmentation ^a	Indicate to:
less than 15%	Typically considered (within the normal range)
Between 15% and 25% or even 30%	Need to take a treatment as a short time
More than 25% or 30%	Need to take a treatment as a long time

^a Reference values for DNA fragmentation may vary from source to source depending on the method of working in the IVF laboratory and the kit used.

chromatin structure. The SCD test evaluates the susceptibility of sperm DNA to acid-induced denaturation. During the experiment, the sperm cells are mixed with an acid solution, causing the sperm DNA to relax and denature. Denatured DNA fragments are then stained with a fluorescent dye, and the resulting sperm cells are examined under a microscope.

In this method, before preparing the semen sample for artificial insemination, 500 sperm cells were examined under the light microscope (Fig. 1) before applying the sample preparation steps. According to the detailed method from the manufacturer (<http://www.spermfunc.com/>), 60 µL are taken from the sample and mixed by taking 30 µL from the gel in the tube. It is then spread on a slide and placed in the refrigerator, followed by 7 min (Denaturation solution) and then 25 min (Lysis solution). The sample is washed with distilled water, followed by placing the sample at 70%, 90%, and 100% concentrations for each concentration for 2 min, respectively, followed by fifteen drops of Wright's solution and then thirty drops of Wright's buffer. The sample is then washed with distilled water, left to dry, and examined at 40× magnification (Al-Darawsha et al., 2023).

In Picture (A), we observe Non-Halo Sperm, which typically indicates a lack of dispersion or limited DNA fragmentation. These sperm cells do not exhibit the characteristic halo pattern around the nucleus when subjected to chromatin dispersion testing. Conversely, but in picture (B), we observe Halo sperm, where a distinct halo-like structure is evident around the sperm nucleus. This halo pattern indicates successful chromatin dispersion and is associated with lower levels of DNA fragmentation. Such sperm cells are considered to have more intact DNA.

Aniline blue staining method

Aniline blue staining is a laboratory technique used to evaluate the maturity and quality of sperm cells, particularly sperm chromatin integrity and the presence of abnormal sperm chromatin packaging. In normal, mature sperm cells, chromatin is tightly packed and does not bind to Aniline blue stain. However, in sperm cells with immature or abnormal chromatin, the dye will bind to histones, causing a blue coloration in these cells. In our study, 200 cells were counted for each sample before starting the semen sample for IUI. According to the manufacturer's (<http://www.spermfunc.com/>) detailed method, we take the steps starting with taking two hundred microliters of sample and mixing it with one ml of

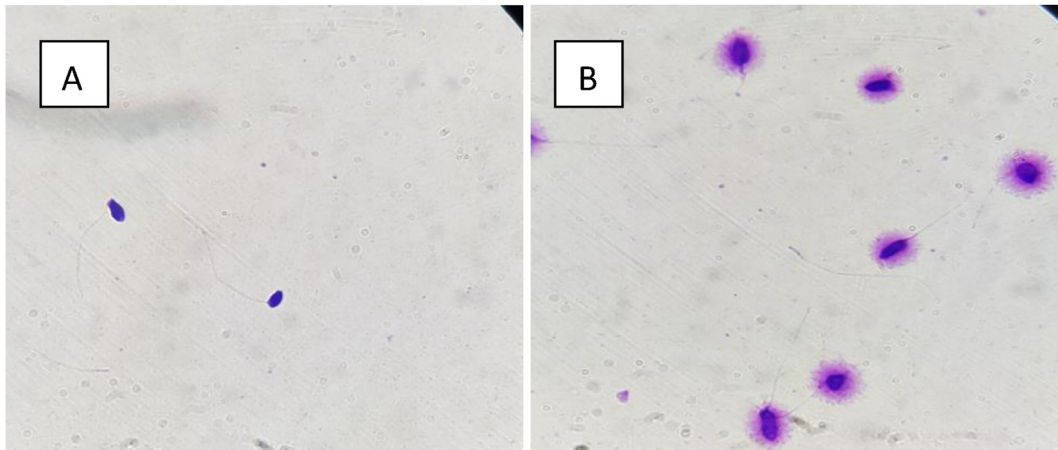


Fig. 1. The images presented sperm chromatin dispersion, where (A) represents non-Halo sperm and (B) depicts Halo sperm.

distilled water, then we get the sediment. centrifuged and a hundred milliliters of solution (Sperm washing medium) is placed on the precipitate and mixed thoroughly, and then 5 μ L are taken from the mixture and applied to the slide until dry and a fixative solution (10% formaldehyde) is placed on the sample and left for 30 min, then it is washed and left to dry, then solution C (Aniline blue stain) is placed and left for 5 min and the sample is dried after washing with distilled water. The sample is kept vertically in solution D (Elution(HCL)) for 5 min, and after washing and drying the sample, adding solution E (Xanthene dye) ((xanthene and Sodium azide)) and waiting for 5 min, then washed. It is dried, examined under a light microscope (Fig. 2) under an oil of 100x (Al-Darawsha et al., 2023).

In this picture, the sperm heads appear with a distinct cyaneous or bluish tint when subjected to staining. This staining pattern highlights the

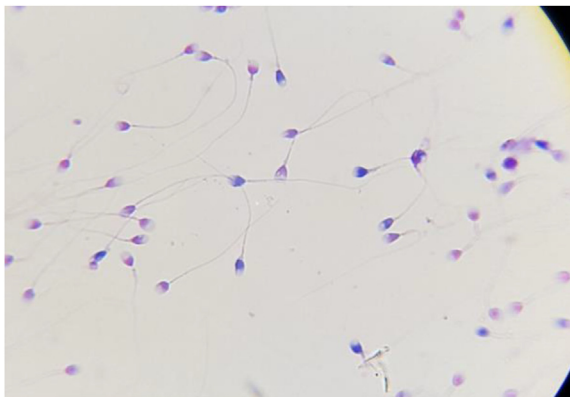


Fig. 2. The images presented here showcase sperm cells with cyaneous-stained heads, which is indicative of sperm containing immature nucleoproteins.

presence of nucleoproteins in the sperm's nucleus that are not fully matured or developed. The cyaneous-stained heads are a visual indicator of the sperm's chromatin quality, particularly in terms of nucleoprotein maturation.

Sperm preparation for intrauterine insemination

The goal of sperm preparation is to select motile, high-quality sperm and remove any impurities or debris from the semen sample. There are many methods used in IVF centers and the most used is Swim-Up Technique, this method is commonly used in IVF centers and involves placing the semen sample on top of a culture medium and allowing the motile sperm to swim up to the surface over a specific period, usually around 30 min to an hour. The motile sperm are then collected from the upper layer and used for insemination. Sperm preparation for intrauterine insemination (IUI) involves several steps to optimize the quality and concentration of sperm before the insemination procedure. These include, Semen Processing: Semen processing typically involves the following steps: a. Semen Liquefaction: If the semen sample is clotted or not in a liquid state, it is allowed to liquefy at room temperature for about 20–30 min. b. Semen Dilution: The semen sample is diluted with a specially formulated medium, such as Ham's F-10 or HTF (Human Tubal Fluid), which provides optimal conditions for sperm survival and motility. Dilution helps reduce the concentration of seminal plasma and allows for better sperm mobility. c. Sperm Wash: The diluted semen is then subjected to a process called sperm washing. It involves centrifugation, which separates the sperm cells from the seminal plasma and any debris or non-sperm

components. After centrifugation, the supernatant (liquid portion) is discarded, and the sperm pellet is retained. d. Sperm Re suspension: The sperm pellet obtained from the washing step is re suspended in a smaller volume of the prepared medium. This step concentrates the sperm cells, making it easier to calculate the desired sperm concentration for the IUI procedure. e. Sperm Concentration Adjustment: The prepared sperm suspension is then adjusted to achieve the desired sperm concentration. f. Insemination: Once the sperm preparation is complete, the prepared sperm is loaded into a catheter or syringe and introduced into the woman's uterus through the cervix during the Intra Uterine Insemination procedure (Davies & Cumming, 1999).

Statistical analysis

The statistical analysis was performed using the non-parametric (Spearman's rho) correlation between SCD and ABS with each other was used in the statistical analysis (male age, body mass index, progressive sperm motility, sperm morphology, and sperm concentration). All statistical analyses were performed using the Statistical Package for the Social Sciences software version 26.0 (IBM Corporation, Armonk, NY, USA).

Results

In our study in Iraq-Baghdad city, 111 males who met the inclusion criteria were recruited for our study. Selected participants were grouped according to their age, body mass index, and semen parameters, with aniline blue staining and sperm chromatin dispersion staining. Arithmetical means, standard deviations, and percentage rates were used to present the data in Table 4.

In Table 5 In our study, When we examined the correlations between aniline blue stain and various factors, including male age ($r = 0.08$, $p = 0.382$), body mass index (BMI) ($r = 0.112$, $p = 0.238$), sperm concentration ($r = -0.149$, $p = 0.117$), sperm morphology ($r = -0.115$, $p = 0.229$), and sperm motility ($r = -0.166$, $p = 0.079$), we found that these

Table 5. Correlation analysis of Aniline blue stain with Male age, BMI and Sperm parameters.

Parameters	Aniline blue staining	
	Correlation coefficient	Sig. (2-tailed)
Male age	0.084	0.382
BMI	0.113	0.238
Sperm concentration	-0.150	0.117
Sperm morphology	-0.115	0.229
Sperm motility	-0.167	0.080

*Correlation is significant at the 0.05 level (2-tailed).

correlations did not yield statistically significant associations. These results suggest that, within our study, aniline blue stain did not demonstrate a strong relationship with male age, BMI, sperm concentration, sperm morphology, or sperm motility.

In Table 6 when we conducted correlation analyses to examine the relationships between Sperm chromatin dispersion staining and various factors, including male age ($r_s = 0.155$, $p = 0.104$), body mass index ($r_s = 0.155$, $p = 0.102$), and sperm motility ($r_s = -0.130$, $p = 0.173$), we observed that these variables did not exhibit statistically significant associations with sperm chromatin dispersion staining. However, when we explored the correlations with sperm concentration ($r_s = -0.212$; $p = 0.025$) and sperm morphology ($r_s = -0.196$; $p = 0.039$), we found a related relationship between these parameters and sperm chromatin dispersion staining. This implies that, within our study, sperm chromatin dispersion staining demonstrated a measurable association with sperm concentration

Table 6. Sperm chromatin dispersion relationships with Male age, BMI, Sperm concentration, Sperm morphology and Sperm motility.

Parameters	Sperm chromatin dispersion	
	Correlation coefficient	Sig. (2-tailed)
Male age	0.173	0.070
BMI	0.156	0.103
Sperm concentration	-0.212 ^a	0.025
Sperm morphology	-0.196 ^a	0.039
Sperm motility	-0.130	0.173

^a Correlation is significant at the 0.05 level (2-tailed).

Table 4. The Lower, Upper and Average limits for the data collected during our study period.

Parameters	Number of patients	Average	Median	Mode	Minimum	Maximum	Standard deviation
Male age	N: 111	35	36	30	23	44	4.37
Body mass index		25.24	24.53	26.75	13.88	42.66	6.44
Sperm concentration (million/ml)		64.70	54	59	25	190	36.07
Sperm morphology (%)		22%	23%	19%	17%	30%	4%
Sperm motility (%)		37%	37%	33%	25%	69%	8%
Aniline blue staining (%) (ABS)		41%	43%	45%	14%	67%	12%
Halo sperm staining (%) (SCD)		31%	31%	30%	9%	51%	11%

and sperm morphology, whereas it did not appear to be strongly related to male age, BMI, or sperm motility.

Discussion

Understanding the causes, diagnosis, and treatment options for male infertility is crucial in addressing this widespread issue, and male infertility contributes to nearly 40% of all infertility cases (Krausz, 2011). With advancements in medical technology and treatment options, there is hope for couples facing male infertility to achieve their dream of parenthood (Franklin, 1990). Diagnosing male infertility typically involves a comprehensive evaluation of the man's medical history, physical examination, semen analysis, and maybe body mass index and old age (Barratt et al., 2017).

Body mass index, age, and sperm parameters are essential for evaluating male infertility (Sekhavat & Moein, 2010). Research has shown that these factors can have significant implications for sperm quality, reproductive function, and the chances of achieving pregnancy (Sharma et al., 2015). Body mass index is a numerical measure of an individual's body fat calculated from their weight and height (Akindele et al., 2016). It plays a significant role in overall health, and research has shown that it can have a notable impact on male fertility (Adewoyin et al., 2017). Studies have indicated that both low and high BMI levels can lead to fertility issues (Katib, 2015). This can lead to a lower sperm count and decreased fertility (Rodprasert et al., 2022). Men with a low BMI may have reduced sperm production due to hormonal imbalances, particularly lower testosterone levels (Santi et al., 2023). These hormonal imbalances can adversely affect sperm quality, motility, and overall fertility (Ameratunga et al., 2023). Excess body fat can result in hormonal disturbances, including increased estrogen production and decreased testosterone levels (Khodamoradi et al., 2022). Elevated levels of sperm DNA fragmentation can reduce the sperm's ability to fertilize an egg and may lead to difficulties in achieving pregnancy (Khallaf et al., 2022). Higher BMI levels in men undergoing ART have been associated with lower pregnancy rates and increased chances of IVF failure (Imterat et al., 2019). High BMI has also been linked to an increased risk of sperm DNA fragmentation (Agarwal et al., 2020). BMI can influence the success rates of Assisted Reproductive Technologies (ART), such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (Chen et al., 2022).

Both underweight and overweight or obese conditions can have adverse effects on sperm production, quality, and DNA integrity (Salas-Huetos et al., 2021). Studies have suggested a correlation between BMI and sperm DNA fragmentation. Low and high BMI levels have been associated with increased sperm DNA damage (Chavarro et al., 2010b). On the other hand, obesity, characterized by a high BMI, is linked to oxidative stress, inflammation, and hormonal imbalances, which can contribute to sperm DNA damage (Bondia-Pons et al., 2012). Conversely, low BMI, particularly in underweight individuals, may also adversely affect sperm DNA integrity (Sharma et al., 2013).

In the course of our study, a thorough analysis of the data revealed an interesting observation: we did not identify any statistically significant correlation between aniline blue staining (ABS), sperm chromatin damage (SCD), and body mass index (BMI), and this finding underscores the complexity of the factors influencing male fertility, as BMI, in this instance, did not appear to have a discernible impact on these specific parameters within our study.

Age is another crucial factor in male infertility; as men age, the quality and quantity of sperm tend to decline (Kumar & Singh, 2015b). Advanced paternal age is associated with increased DNA fragmentation in sperm, decreased sperm motility, and higher rates of chromosomal abnormalities (Das et al., 2013). Furthermore, advanced paternal age has been associated with increased sperm DNA fragmentation in older men age; there is a higher likelihood of accumulated DNA damage in sperm cells, which can lead to increased fragmentation (Puscheck & Jeyendran, 2007). Age-related factors such as increased oxidative stress decreased DNA repair mechanisms, and gradual decline in overall sperm quality contribute to this relationship (Liang & Godley, 2003). The reasons for this phenomenon are multi-factorial and include factors such as oxidative stress, reduced DNA repair mechanisms, and lifestyle factors (Ziolkowska et al., 2021). Advanced paternal age, typically considered to be above 50 years, has indeed been associated with an increased risk of sperm DNA fragmentation (du Fosse et al., 2020). As men age, there is a higher likelihood of accumulated DNA damage in sperm cells, which can lead to increased fragmentation (Panner Selvam et al., 2021). The presence of DNA fragmentation in sperm can have implications for fertility, as it can affect the sperm's ability to fertilize an egg and may also increase the risk of genetic abnormalities in offspring. This is an important consideration for

couples when planning for parenthood at an older age (Bashiri et al., 2021).

Through an examination of our study data, we did not detect a statistically significant correlation between aniline blue staining (ABS), a measure of sperm chromatin integrity, sperm chromatin damage (SCD), and the increasing age of male participants. And this finding, drawn from our study, suggests that age, in the context of our research, did not appear to exert a discernible influence on the relationship between ABS, SCD, and male aging.

On the other hand, Sperm parameters, including Sperm count, Motility, Morphology, and DNA fragmentation, play a significant role in male fertility (Cohen-Bacrie et al., 2009b). Maybe abnormalities in these parameters can impact the ability of sperm to fertilize an egg and achieve a successful pregnancy (Sakkas et al., 1996). Some studies indicated abnormalities in these parameters, such as low sperm count, poor motility, and abnormal morphology, have been associated with increased DNA fragmentation (Khadem et al., 2014). As well as It is also believed that sperm with poor quality or function may be more susceptible to DNA damage (Agarwal & Said, 2003). Others several research studies have provided evidence suggesting that abnormalities in fundamental sperm parameters, including low sperm count, diminished motility, and irregular sperm morphology, have shown associations with heightened levels of DNA fragmentation (Birowo et al., 2020). These findings indicate that compromised sperm quantity, quality, and structural integrity may contribute to an elevated risk of DNA fragmentation within sperm cells (Pino et al., 2020b). This interplay between sperm parameters and DNA fragmentation underscores the complexity of male infertility and its multifaceted factors (Tahmasbpour et al., 2014).

In our study, we did not uncover any statistically significant correlation between ABS with sperm motility, sperm concentration, and the morphology of sperm cells. But, based on our study, highlights that SCD demonstrated a clear and measurable association with both sperm concentration and the morphology of sperm cells. These correlations provide valuable insights into the relationships between SCD, sperm concentration, and sperm morphology within the context of our research.

Through our results in this research and the study of a group of research, we can conclude that there may not be a relationship between BMI and age, but sperm parameters with sperm DNA fragmentation may be related underscoring the importance of evaluating and addressing multiple aspects of male fertility when assessing infertility. For men

approaching or beyond middle age, it is essential to be aware of the potential effects of sperm DNA damage on fertility including sperm concentration and sperm motility, and consider seeking fertility evaluations semen analysis together with sperm DNA damage, and treatments if needed.

Conclusion

Based on the findings from this study and a body of related research, it becomes apparent that BMI and age may not exhibit a direct relationship. However, the noteworthy association between sperm parameters and sperm DNA fragmentation underscores the significance of a comprehensive evaluation of male fertility when dealing with infertility issues. Especially for men entering middle age or beyond, it is crucial to acknowledge the potential implications of sperm DNA damage on fertility, including its impact on sperm concentration and motility. In such cases, it is advisable to consider fertility assessments, including semen analysis coupled with sperm DNA damage evaluation, and explore appropriate treatments when necessary. In response to these considerations, the integration of male fertility screening and prognostic assessments has gained growing importance within IVF centers.

Recommendation

- 1 Incorporate sperm DNA fragmentation testing: Prior to commencing assisted reproductive Technology (ART) procedures, it is imperative to conduct thorough sperm DNA fragmentation testing within infertility centers.
- 2 Comprehensive evaluation of male factors: When evaluating and managing male infertility, it is vital to take into account several key factors. These include body mass index (BMI), age, and various sperm parameters.
- 3 Prioritize health and age considerations: A crucial aspect of male fertility management is the maintenance of a healthy BMI. Additionally, recognizing age-related changes is paramount, as these factors can significantly impact fertility outcomes.
- 4 Sperm parameters: Addressing any abnormalities in sperm parameters is equally essential, as it can play a pivotal role in enhancing sperm quality and overall fertility potential.

Ethics approval and consent to participate

Not need approval.

Consent for publication

Acceptance for publication.

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Authors' contributions

The author tries to make a substantial and intellectual contribution to the subject research topic.

Conflict of interest

The author declares that there is no conflict of interest.

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No.

References

- Adewoyin, M., Ibrahim, M., Roszaman, R., Md Isa, M. L., Mat Alewi, N. A., Abdul Rafa, A. A., & Anuar, M. N. (2017 Mar 1). Male infertility: The effect of natural antioxidants and phytochemicals on seminal oxidative stress. *Diseases*, 5(1), 9.
- Agarwal, A., Majzoub, A., Baskaran, S., Selvam, M. K., Cho, C. L., Henkel, R., Finelli, R., Leisegang, K., Sengupta, P., Barbarosie, C., & Parekh, N. (2020 Oct). Sperm DNA fragmentation: A new guideline for clinicians. *The World Journal of Men's Health*, 38(4), 412.
- Agarwal, A., & Said, T. M. (2003 Jul 1). Role of sperm chromatin abnormalities and DNA damage in male infertility. *Human Reproduction Update*, 9(4), 331–345.
- Akindele, M. O., Phillips, J. S., & Igumbor, E. U. (2016 Aug 8). The relationship between body fat percentage and body mass index in overweight and obese individuals in an urban African setting. *Journal of Public Health in Africa*, 7(1).
- Akingbemi, B. T. (2005 Dec). Estrogen regulation of testicular function. *Reproductive Biology and Endocrinology*, 3, 1–3.
- Al-Darawsha, T. Z., Dayioglu, N., Al-Azzawi, B. R., & Irez, T. (2023 Jan 31). Can the prediction of intrauterine insemination results by used aniline blue stain (ABS) and sperm chromatin dispersion (SCD) levels? *Advances in Reproductive Sciences*, 11(1), 1-0.
- Ameratunga, D., Gebeh, A., & Amoako, A. (2023 Aug 4). Obesity and male infertility. *Best Practice & Research Clinical Obstetrics & Gynaecology*, Article 102393.
- Ammar, O., Haouas, Z., Hamouda, B., Hamdi, H., Hellara, I., Jlali, A., Cheikh, H. B., & Mehdi, M. (2019 Feb 1). Relationship between sperm DNA damage with sperm parameters, oxidative markers in teratozoospermic men. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 233, 70–75.
- Barratt, C. L., Björndahl, L., De Jonge, C. J., Lamb, D. J., Osorio Martini, F., McLachlan, R., Oates, R. D., van der Poel, S., St John, B., Sigman, M., & Sokol, R. (2017 Nov 1). The diagnosis of male infertility: An analysis of the evidence to support the development of global WHO guidance—challenges and future research opportunities. *Human Reproduction Update*, 23(6), 660–680.
- Bashiri, Z., Amidi, F., Amiri, I., Zandieh, Z., Maki, C. B., Mohammadi, F., Amiri, S., & Koruji, M. (2021 Jul). Male factors: The role of sperm in preimplantation embryo quality. *Reproductive Sciences*, 28, 1788–1811.
- Birowo, P., Rahendra Wijaya, J., Atmoko, W., & Rasyid, N. (2020 Dec). The effects of varicocele on the DNA fragmentation index and other sperm parameters: A meta-analysis. *Basic and Clinical Andrology*, 30, 1–9.
- Bondia-Pons, I., Ryan, L., & Martinez, J. A. (2012 Dec). Oxidative stress and inflammation interactions in human obesity. *Journal of Physiology & Biochemistry*, 68, 701–711.
- Caballero, B. (2019 Jan 1). Humans against obesity: Who will win? *Advances in Nutrition*, 10(suppl. 1), S4–S9.
- Chan, P. T., & Robaire, B. (2022 Jun 9). Advanced paternal age and future generations. *Frontiers in Endocrinology*, 13, Article 897101.
- Chavarro, J. E., Toth, T. L., Wright, D. L., Meeker, J. D., & Hauser, R. (2010a). Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. *Fertility and Sterility*, 93(7), 2222–2231.
- Chavarro, J. E., Toth, T. L., Wright, D. L., Meeker, J. D., & Hauser, R. (2010b). Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. *Fertility and Sterility*, 93(7), 2222–2231.
- Chen, H., Li, J., Cai, S., Zeng, S., Yin, C., Kuang, W., Cheng, K., Jiang, Y., Tao, M., Chu, C., & Hoher, J. G. (2022 Jan). Impact of body mass index (BMI) on the success rate of fresh embryo transfer in women undergoing first in vitro fertilization/ intracytoplasmic sperm injection (IVF/ICSI) treatment. *International Journal of Obesity*, 46(1), 202–210.
- Cohen-Bacrie, P., Belloc, S., Ménézo, Y. J., Clement, P., Hamidi, J., & Benkhalifa, M. (2009a). Correlation between DNA damage and sperm parameters: A prospective study of 1,633 patients. *Fertility and Sterility*, 91(5), 1801–1805.
- Cohen-Bacrie, P., Belloc, S., Ménézo, Y. J., Clement, P., Hamidi, J., & Benkhalifa, M. (2009b). Correlation between DNA damage and sperm parameters: A prospective study of 1,633 patients. *Fertility and Sterility*, 91(5), 1801–1805.
- Das, M., Al-Hathal, N., San-Gabriel, M., Phillips, S., Kadoch, I. J., Bissonnette, F., Holzer, H., & Zini, A. (2013 Jun). High prevalence of isolated sperm DNA damage in infertile men with advanced paternal age. *Journal of Assisted Reproduction and Genetics*, 30, 843–848.
- Davies, N. J., & Cumming, D. C. (1999 Dec 1). Sperm preparation for intra-uterine insemination. *Journal SOG*, 21(14), 1294–1303.
- Du Plessis, S. S., Cabler, S., McAlister, D. A., Sabanegh, E., & Agarwal, A. (2010 Mar). The effect of obesity on sperm disorders and male infertility. *Nature Reviews Urology*, 7(3), 153–161.
- Esposito, K., Giugliano, F., Di Palo, C., Giugliano, G., Marfella, R., D'Andrea, F., D'Armiento, M., & Giugliano, D. (2004 Jun 23). Effect of lifestyle changes on erectile dysfunction in obese men: A randomized controlled trial. *JAMA*, 291(24), 2978–2984.
- Evenson, D. P. (2016 Jun 1). The Sperm Chromatin Structure Assay (SCSA®) and other sperm DNA fragmentation tests for evaluation of sperm nuclear DNA integrity as related to fertility. *Animal Reproduction Science*, 169, 56–75.
- Evgeni, E., Charalabopoulos, K., & Asimakopoulos, B. (2014 Jan). Human sperm DNA fragmentation and its correlation with conventional semen parameters. *Journal of Reproduction and Infertility*, 15(1), 2.
- du Fosse, N. A., Van der Hoorn, M. L., van Lith, J. M., le Cessie, S., & Lashley, E. E. (2020 Sep 1). Advanced paternal age is associated with an increased risk of spontaneous miscarriage: A systematic review and meta-analysis. *Human Reproduction Update*, 26(5), 650–669.
- Franklin, S. (1990). Deconstructing 'desperateness': The social construction of infertility in popular representations of new reproductive technologies. *The New Reproductive Technologies*, 200–229.
- Hammiche, F., Laven, J. S., Boxmeer, J. C., Dohle, G. R., Steegers, E. A., & Steegers-Theunissen, R. P. (2011 Jan 2). Sperm quality decline among men below 60 years of age undergoing IVF or ICSI treatment. *Journal of Andrology*, 32(1), 70–76.
- Hammoud, A. O., Gibson, M., Peterson, C. M., Hamilton, B. D., & Carrell, D. T. (2006 Sep 10). Obesity and male reproductive potential. *Journal of Andrology*, 27(5), 619–626.

- Imterat, M., Agarwal, A., Esteves, S. C., Meyer, J., & Harlev, A. (2019 Mar 1). Impact of body mass index on female fertility and ART outcomes. *Panminerva Medica*, 61(1), 58–67.
- Kaltsas, A., Moustakli, E., Zikopoulos, A., Georgiou, I., Dimitriadis, F., Symeonidis, E. N., Markou, E., Michaelidis, T. M., Tien, D. M., Giannakis, I., & Ioannidou, E. M. (2023 Feb 14). Impact of advanced paternal age on fertility and risks of genetic disorders in offspring. *Genes*, 14(2), 486.
- Katib, A. (2015). Mechanisms linking obesity to male infertility. *Central European journal of urology*, 68(1), 79.
- Khadem, N., Poorhoseyni, A., Jalali, M., Akbary, A., & Heydari, S. T. (2014 Mar). Sperm DNA fragmentation in couples with unexplained recurrent spontaneous abortions. *Andrologia*, 46(2), 126–130.
- Khallaf, M. Y., Abdelatif, S. B., Said, A. M., & Helal, H. A. (2022 Oct 28). The influence of sperm DNA fragmentation on blastulation and implantation rates in different sources of sperms. *Egyptian Academic Journal of Biological Sciences, B. Zoology*, 14(2), 299–313.
- Khodamoradi, K., Khosravizadeh, Z., Seetharam, D., Mallepalli, S., Farber, N., & Arora, H. (2022 Nov). The role of leptin and low testosterone in obesity. *International Journal of Impotence Research*, 34(7), 704–713.
- Krausz, C. (2011 Apr 1). Male infertility: Pathogenesis and clinical diagnosis. *Best Practice & Research Clinical Endocrinology & Metabolism*, 25(2), 271–285.
- Kumar, N., & Singh, A. K. (2015a). Trends of male factor infertility, an important cause of infertility: A review of literature. *Journal of Human Reproductive Sciences*, 8(4), 191.
- Kumar, N., & Singh, A. K. (2015b). Trends of male factor infertility, an important cause of infertility: A review of literature. *Journal of Human Reproductive Sciences*, 8(4), 191.
- Le, M. T., Nguyen, T. A., Nguyen, H. T., Nguyen, T. T., Nguyen, V. T., Le, D. D., Nguyen, V. Q., & Cao, N. T. (2019 Oct). Does sperm DNA fragmentation correlate with semen parameters? *Reproductive Medicine and Biology*, 18(4), 390–396.
- Liang, F. Q., & Godley, B. F. (2003 Apr 1). Oxidative stress-induced mitochondrial DNA damage in human retinal pigment epithelial cells: A possible mechanism for RPE aging and age-related macular degeneration. *Experimental Eye Research*, 76(4), 397–403.
- Panner Selvam, M. K., Ambar, R. F., Agarwal, A., & Henkel, R. (2021 Feb). Etiologies of sperm DNA damage and its impact on male infertility. *Andrologia*, 53(1), Article e13706.
- Patel, A. P., Patel, J. A., Cruz, M., Gupte-Shah, A., Garcia Velasco, J. A., & Banker, M. R. (2016 Oct 2). Ethnicity is an independent predictor of IVF-ICSI outcome: A study of 5,549 cycles in Spain and India. *Gynecological Endocrinology*, 32(10), 819–822.
- Petersen, C. G., Mauri, A. L., Vagnini, L. D., Renzi, A., Petersen, B., Mattila, M., Comar, V., Ricci, J., Dieamant, F., Oliveira, J. B., & Baruffi, R. L. (2018 Oct). The effects of male age on sperm DNA damage: An evaluation of 2,178 semen samples. *JBRA Assisted Reproduction*, 22(4), 323.
- Pino, V., Sanz, A., Valdés, N., Crosby, J., & Mackenna, A. (2020a). The effects of aging on semen parameters and sperm DNA fragmentation. *JBRA Assisted Reproduction*, 24(1), 82.
- Pino, V., Sanz, A., Valdés, N., Crosby, J., & Mackenna, A. (2020b). The effects of aging on semen parameters and sperm DNA fragmentation. *JBRA Assisted Reproduction*, 24(1), 82.
- Plastira, K., Msaouel, P., Angelopoulou, R., Zanioti, K., Plastiras, A., Pothos, A., Bolaris, S., Papanisteidis, N., & Mantas, D. (2007 Oct). The effects of age on DNA fragmentation, chromatin packaging and conventional semen parameters in spermatozoa of oligoasthenoteratozoospermic patients. *Journal of Assisted Reproduction and Genetics*, 24, 437–443.
- Puschek, E. E., & Jeyendran, R. S. (2007 Jun 1). The impact of male factor on recurrent pregnancy loss. *Current Opinion in Obstetrics and Gynecology*, 19(3), 222–228.
- Rodprasert, W., Toppari, J., & Virtanen, H. E. (2022 Dec 17). Environmental toxicants and male fertility. *Best Practice & Research Clinical Obstetrics & Gynaecology*, Article 102298.
- Rosiak-Gill, A., Gill, K., Jakubik, J., Fraczek, M., Patorski, L., Gaczarzewicz, D., Kurzawa, R., Kurpisz, M., & Piasecka, M. (2019 Aug 8). Age-related changes in human sperm DNA integrity. *Aging (Albany NY)*, 11(15), 5399.
- Sakkas, D., Urner, F., Bianchi, P. G., Bizzaro, D., Wagner, I., Jaquenoud, N., Manicardi, G., & Campana, A. (1996 Apr 1). Sperm chromatin anomalies can influence decondensation after intracytoplasmic sperm injection. *Human Reproduction*, 11(4), 837–843.
- Salas-Huetos, A., Maghsoumi-Norouzabad, L., James, E. R., Carrell, D. T., Aston, K. I., Jenkins, T. G., Becerra-Tomás, N., Javid, A. Z., Abed, R., Torres, P. J., & Luque, E. M. (2021 Jan). Male adiposity, sperm parameters and reproductive hormones: An updated systematic review and collaborative meta-analysis. *Obesity Reviews*, 22(1), Article e13082.
- Santi, D., Lotti, F., Sparano, C., Rastrelli, G., Isidori, A. M., Pivonello, R., Barbonetti, A., Salonia, A., Minhas, S., Krausz, C., & Vignozzi, L. (2023 May 25). Does an increase in adipose tissue 'weight' affect male fertility? A systematic review and meta-analysis based on semen analysis performed using the WHO 2010 criteria. *Andrology*, 13460, 1–14. <https://doi.org/10.1111/andr.13460>
- Sekhavaṭ, L., & Moein, M. R. (2010 Sep 1). The effect of male body mass index on sperm parameters. *The Aging Male*, 13(3), 155–158.
- Seriki SA, Mfem CC, Oyama SE, Otoikhila OC. Impact of age and diet on semen quality and male fertility.
- Sharma, R., Agarwal, A., Rohra, V. K., Assidi, M., Abu-Elmagd, M., & Turki, R. F. (2015 Dec). Effects of increased paternal age on sperm quality, reproductive outcome and associated epigenetic risks to offspring. *Reproductive Biology and Endocrinology*, 13, 1–20.
- Sharma, R., Biedenharn, K. R., Fedor, J. M., & Agarwal, A. (2013 Dec). Lifestyle factors and reproductive health: Taking control of your fertility. *Reproductive Biology and Endocrinology*, 11(1), 1–5.
- Sheykh N, Amiri I, Farimani M, Najafi R, Hadiei J. Correlation between sperm parameters and sperm DNA fragmentation in fertile and infertile men.
- Skakkebaek, N. E., Lindahl-Jacobsen, R., Levine, H., Andersson, A. M., Jørgensen, N., Main, K. M., Ø, L., Priskorn, L., Holmboe, S. A., Bräuner, E. V., & Almstrup, K. (2022 Mar). Environmental factors in declining human fertility. *Nature Reviews Endocrinology*, 18(3), 139–157.
- Stone, B. A., Alex, A., Werlin, L. B., & Marrs, R. P. (2013 Oct 1). Age thresholds for changes in semen parameters in men. *Fertility and Sterility*, 100(4), 952–958.
- Tahmasbpour, E., Balasubramanian, D., & Agarwal, A. (2014 Sep). A multi-faceted approach to understanding male infertility: Gene mutations, molecular defects and assisted reproductive techniques (ART). *Journal of Assisted Reproduction and Genetics*, 31, 1115–1137.
- Tandara, M., Bajić, A., Tandara, L., Šunj, M., Jurišić, Z., & Jukić, M. (2013 May 1). Correlation between proportions of sperm with DNA fragmentation assessed by Halosperm test and values of standard quality parameters of semen and possible impact on embryo quality. *Slovenian Medical Journal*, 82(5).
- World Health Organisation. (1999 May 13). *WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction*. Cambridge University Press.
- Yu, X., Zhang, S., Wei, Z., Zhang, X., & Wang, Q. (2022 Jul 28). Prevalence of sexual dysfunction among the male populations who seeking medical care for infertility, pregnancy loss and preconception care: A cross-sectional study. *Scientific Reports*, 12(1), Article 12969.
- Zegers-Hochschild, F., Adamson, G. D., De Mouzon, J., Ishihara, O., Mansour, R., Nygren, K., Sullivan, E., & Van der Poel, S. (2009 Nov 1). The international committee for monitoring assisted reproductive technology (ICMART) and the world health organization (WHO) revised glossary on ART terminology. *Human Reproduction*, 24(11), 2683–2687.
- Ziolkowska, S., Binienda, A., Jabikowski, M., Szemraj, J., & Czarny, P. (2021 Oct 15). The interplay between insulin resistance, inflammation, oxidative stress, base excision repair and metabolic syndrome in nonalcoholic fatty liver disease. *International Journal of Molecular Sciences*, 22(20), Article 11128.