Association between DNA fragmentation index and sperm functional characteristics among men from infertile couples in Abuja, Nigeria

Anthony Ilegogie[1,2] and Mathias A Emokpae[1]

[1] Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City. Nigeria
[2]Department of Chemical Pathology, National Hospital, Abuja, Nigeria.

Abstract

Background
Semen analysis is still the bedrock on which the evaluation of male infertility is based, but the parameters of conventional semen analysis do not reliably predict neither male fertility nor provide information on DNA integrity, which is one of the most important components of the reproductive outcome. This study seeks to evaluate the proportion of sperm DNA fragmentation index and its association with sperm functional characteristics and the age of men from infertile couples investigated for infertility.
Materials and Methods: Semen analysis and sperm DNA fragmentation index (SDFI) were determined in infertile males and fertile control subjects using the SQAV sperm quality analyzer and TUNEL (terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end labelling) respectively. The chi square, unpaired Student's t-test, and analysis of variance (ANOVA) were used to compare the means between the groups, while Pearson's correlation coefficient was used to test the association between SDFI, sperm functional characteristics and age of the subjects.
Results and Discussion: The SDFI in infertile men, 66.4±2.6%, was significantly higher ($p<0.001$) than 16.0±1.1% in fertile control subjects. The SDFI in infertile subjects with normozoospermia was 44.2±2.7%, mild oligozoospermia 65.4±2.4% and severe oligozoospermia 60.2±2.4% respectively. The SDFI inversely correlated with total sperm concentration ($r=-0.76$, $p<0.001$), functional sperm concentration ($r=-0.53$, $p<0.002$) and motile sperm index ($r=-0.58$, $p<0.001$), but an insignificant correlation was observed between SDFI and motile sperm concentration ($r=-0.14$, $p>0.05$). The percentage SDFI increased with increasing age of infertile men, while the percentage of semen samples without sperm DNA fragmentation index decreased with increasing age of infertile males. The SDFI was higher in ejaculates from infertile men than control subjects, which was higher in oligozoospermic than normozoospermic infertile males, and increased with increasing age of infertile subjects.

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Correspondence: M.A. Emokpae **; Email:** mathias.emokpae@uniben.edu
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Introduction

Semen parameters such as concentration, motility, and morphology are commonly used to determine the fertilization potential of sperm during laboratory investigation of male infertility. Although this provides a general overview of the quality of sperm, it does not provide information on one of the most important components of the reproductive outcome, DNA integrity. It is known that DNA damage may involve single-stranded breaks or "nicks," double-stranded breaks or "fragments," deletions/additions, and base modifications. The term DNA fragmentation is technically associated with endonuclease-mediated double-stranded DNA cleavage as a result of several factors. However, it has also become interchangeable with the general term "DNA damage" when in the context of terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end

labelling (TUNEL) assay results (Sharma *et al.*, 2016).

Over the past decade, there has been a growing interest in investigating the contribution of sperm nuclear DNA integrity to male factor infertility (Schulte *et al.*, 2010). Some authors have determined the relationship between sperm DNA fragmentation and assisted reproductive technologies (ART) outcomes, and findings indicated a negative effect on sperm quality and fertility (Panner-Selvam and Agarwal, 2018; Simon *et al.*, 2019). Others have suggested that sperm DNA integrity may be a better predictor of male fertility than routine semen analysis (Evenson *et al.*, 1999). Evidence suggests that conflicting findings of the association between outcomes of assisted reproduction technologies (ARTs), male factor infertility and sperm DNA fragmentation is probably due to differences in assay protocols, lack of standardization of methods, and differences in populations size (Enciso *et al.*, 2006; Simon *et al.*, 2017; Cho and Agarwal, 2018; Sun *et al.*, 2018).

Sperm DNA damage was also reported in 8% of men with normal seminal parameters (Zini *et al.*, 2001), while high levels of sperm DNA damage often correlates with poor seminal parameters such as reduced count and motility or abnormal morphology (Lopes et al, 1998; Irvine *et al.*, 2000; Muratori *et al.*, 2000). There is however paucity of reports on sperm DNA fragmentation studies in Nigeria among men investigated for infertility. The International Conference on Population and Development (ICPD) Plan of action, (ICPD 1994), urges countries to establish holistic programs for the prevention and treatment of infertility. This is still an unmet need in sexual and reproductive health programs in Nigeria. This program ought to be addressed as a basic human and reproductive health right of individuals. In recent years, an increasing trend of male infertility has been reported in the so-called infertility belt of sub-Saharan Africa including Nigeria, thereby raising questions about its causes. It is therefore especially important to evaluate the contribution of SDFI to male infertility in our setting where the prevalence of infertility is high (Okonofua, 2000; Uadia and Emokpae, 2015). The objective of this study was to determine whether an association exists between SDFI, Sperm functional characteristics and age of men investigated for infertility in Abuja, Nigeria.

Materials and Methods

Ethics approval

This study was conducted in strict adherence to declaration of Helsinki (DoH) and Health Research Ethics Committee (HREC) guidelines. Strict confidentiality was maintained regarding the information provided by the participants and special codes and serial numbers were assigned to all specimens. Informed consent forms were signed by each participant in the language understood by the participants. The study was approved by the HREC of the National Hospital, Abuja with reference number NHS/EC/072/2016.

Research Setting

Participants were recruited from the National Hospital and the University of Abuja Teaching Hospital, both in Abuja, Nigeria. These hospitals receive clients from all over the country. The study was conducted in the Department of Chemical Pathology and Medical Microbiology Laboratories in the National hospital, Abuja and the University of Abuja Teaching Hospital, Gwagwalada, Abuja.

Study Design

This is a cross-sectional study of men of infertile couples attending the fertility clinics of the hospitals. All men of infertile couples that visited the health facilities under the study scope in Abuja were initially encouraged to participate in the study. However only those who met the inclusion criteria were eventually recruited.

Inclusion criteria:

Thorough physical and medical examinations were conducted on the participants by the attending physicians. Only those who met the inclusion criteria were recruited in the study. They consist of males aged 21-60 years who were referred to the laboratory for semen analyses as part of their investigation for infertility, gave consent, without physical abnormalities or chronic illnesses. Subjects without chronic clinical illnesses and had their babies within the last one year, whose seminal fluid concentrations were over 15 million sperm cells per milliliter according to WHO criteria

(WHO, 2010) were included and used as controls.

Exclusion Criteria

After physical and clinical examinations, individuals with known pathological or congenital conditions such as severe hypertension, diabetes mellitus, sexually transmitted diseases, testicular varicocele, and genital warts were excluded. Also, individuals currently on antioxidant food supplements, smoke cigarettes, and consume alcohol were also excluded due to their high seminal reactive oxygen species levels and possibly low antioxidant activity which might lead to decreased motility and abnormal sperm morphology.

Sample Size Determination

The sample size was calculated using an estimated prevalence of 24% of DNA fragmentation index among men of infertile couples (Marchlewska *et al.*, 2016) and sample size determination formula for health studies by Lwange and Lemeshow,1991. N=Z2(1-P) P/d2.
A total of 294 male subjects evaluated for fertility were included in the study, and 250 healthy men who had fathered a child within the last 12 months were recruited as controls. Semi structured questionnaires were used to collect socio-demographic data of both infertile and control subjects. The questionnaire was administered by trained research assistants at the various centres. Thereafter, the subjects were instructed how to collect a semen specimen after at least 3 days of sexual abstinence and brought to the laboratory immediately.

Sample Collection

Semen samples: Semen specimens were collected by assisted or self-masturbation directly into wide mouthed containers. The use of condom and lubricant was avoided. Semen was collected after 3 to 5 days of sexual abstinence and submitted to the Laboratory less than one hour after collection. Thereafter, semen analysis was carried out on the samples using the SQAV sperm quality analyzer. The semen specimens were then stratified based on sperm concentrations into normozoospermia, oligozoospermia and azoospermia. The DFI assay was done in the normozoospermic (124), mild oligozoospermic (78) and severe oligozoospermic (48) semen samples.

Assessment of DNA Damage

TUNEL (terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end labelling)
DNA fragmentation induced in spermatozoa was assessed using the TdT-mediated-dUTP nick-end labelling free 3'-OH termini of the DNA in an enzymatic reaction with terminal deoxynucleotidyl transferase (TdT), followed by fluorescein labelling with propidium iodide (Heatwole, 1999). In this study, the modified microscopic TUNEL technique first described by Lapes *et al.*, (1998) was performed.

Procedure of the TUNEL Assay

The slides which had been fixed in methanol-glacial acetic acid (3:1) and kept in the -70^{o}C freezer were taken out and stood at room temperature until thawed. After soaking the slides in PBS for 5 minutes at room temperature, sperm cells were permeabilized in permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate). To perform this, 100 µL permeabilization solution was applied to each section in the glass slides and incubated for 30 minutes at room temperature in a humidified chamber. After washing twice with PBS, cells were treated with 20 µL TUNEL reaction mixture, which was prepared by diluting 1 part enzyme solution (FITC-labelled terminal deoxynucleotidyl transferase-TdT) in 9 parts Label solution (Nucleotide mixture), i.e., 10 µL enzyme solution in 90 µL label solution for each sample. The slides were incubated for 1 hour at 37°C and labelled with 50 µL Propidium Iodide (10 pg/mL) for 30 minutes at room temperature in the dark. Slides were rinsed twice in 50 µL PBS buffer for two minutes and mounted in a 1:1 mixture of ProLong Gold antifade reagent (Invitrogen Molecular Probes, Oregon, USA) and glycerol. Stained cells were quantified on Olympus BX51 fluorescence microscope, with a minimum of 300 sperm per slide being assessed using image analysis software (MacProbe V 4.3, Perceptive Scïentific Instruments, League, Texas). DNA fragmentation in sperm cells was evaluated as negative or positive on the basis of the presence or absence of head staining. The percentage of sperm DNA fragmentation was calculated as the number of TUNEL positive nuclei (FITC-labeled, green) per total number of sperm nuclei (Propidium Iodide, red) in approximately 300 cells (TUNEL

positive=TUNEL positive/TUNEL positive + TUNEL negative x 100%). For a positive control; sperm cells were incubated with 3 U/µL DNAse prior to incubation with the TUNEL reagents, and for a negative control the terminal transferase was omitted from the reaction.

Statistical analysis

The mean of duplicate readings of all measurements with a coefficient of variation (CV) <15% were analyzed. Statistical analysis was performed with Statistical Package for Social Sciences software (SPSS for Windows, version 23.0). The results were expressed as means ± standard deviation. Mean values of measured variables were compared between infertile males and controls using chi square, unpaired Student's t-test or one-way analysis of variance (ANOVA) as appropriate. Relationships between SDFI, sperm functional characteristics and age of infertile males were analyzed using Pearson's correlation coefficient. The statistically significant level was set at $p<0.05$.

Results

Table 1 shows the measured socio-demographic parameters of the study participants. The differences in the mean age, educational status, types of job, location of residence, and number of wives were statistically significant ($P<0.001$). The mean age of the infertile men was 41.54 ± 0.62 years, while that of the fertile men was 35.50 ± 0.55 years. The majority (n= 76, 52.0%) of the infertile men were >40 years, while the majority

Table 1: Socio-demographic characteristics of the study population

Social-demographic parameters	Fertile Men	Infertile	
	n(%)=250(46)	n(%)=294(54)	X^2(P)
Age group (yrs)			
<25	16(6.0)	10(3.0)	
20-35	46(18.0)	44(15.0)	0.001
35-40	170(68.0)	88(30.0)	
>40	18(7.0)	152(52.0)	
Educational status			
None	24(10.0)	16(5.0)	
Primary	40(16.0)	42(14.0)	
Secondary	46(18.0)	52(18.0)	0.001
Tertiary	140(56.0)	184(63.0)	
Type of Job			
Civil Service	146(58.0)	178(61.0)	
Business	48(19.0)	76(26.0)	0.001
Unclassified	56(22.0)	40(14.0)	
Location of Residence			
Rural	24(10.0)	48(16.0)	
Urban	226(90.0)	246(84.0)	0.001
Number of wife			
Monogamy	236(94.0)	268(91.0)	0.001
Polygamy	14(6.0)	26(9.0)	

(n=number, values in parenthesis are in percentage)

Fig 1: Percentage distribution of men investigated for infertility

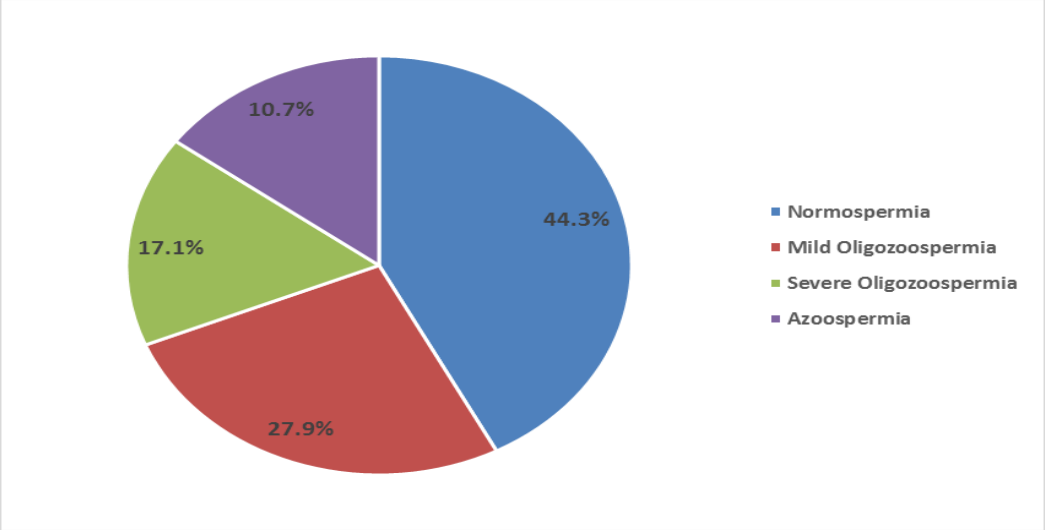


Table 2: Characteristics of sperm indices of infertile subjects

Type	N	%motility	%morphology	%viability
Normozoospermia	124(42.3%)	84(67.7)	96(77.4)	106(85.5)
Mild oligozoospermia	78(26.5%)	50(64.1)	36(46.2)	60(76.9)
Severe oligozoosperm	48(16.3%)	32(66.7)	22(45.8)	36(75.0)
Azoospermia	44(14.9%)	NA	NA	NA

Table 3: The level of sperm fragmentation index based on sperm concentration among infertile subjects and controls.

Variable	Infertile subjects			Fertile subjects
	Normozoospermia Oligozoospermia	Mild oligozoospermia	Severe	
Mean Percentage SDFI	44.2±2.7%	65.4±2.4%)	60.2±2.4%	16.0±1.1%

Fig 2: Percentage distribution of sperm DNA Fragmentation in infertile and fertile subjects

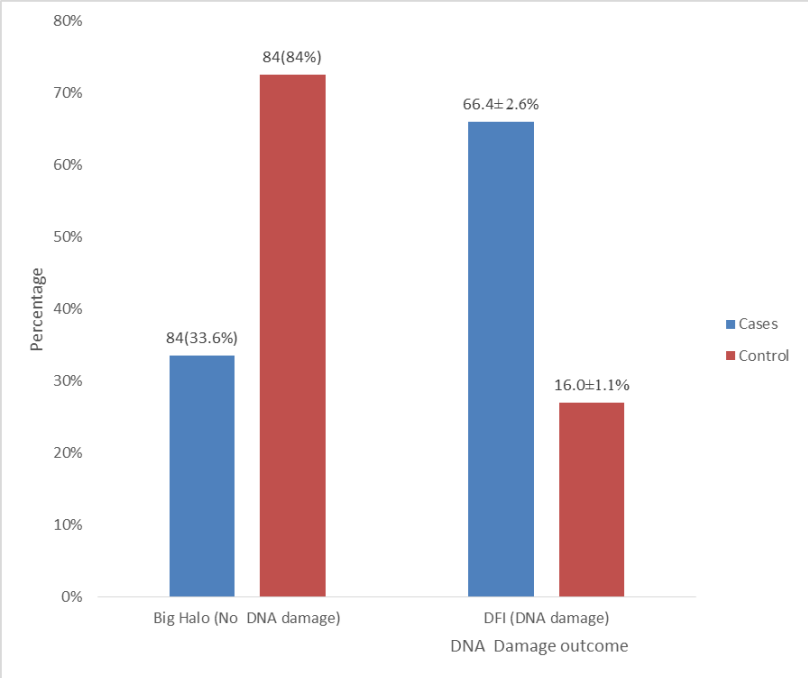


Table 4: Comparison of measured indices of semen quality among infertile subjects based on sperm concentrations

Parameters	Description				
	Normo-spermia Mean± SEM n=124	Mild Oligozoo-spermia Mean± SEM n=78	Severe Oligozoo-spermia Mean± SEM n=48	Azoo-spermia (n=44)	p-value
TSC	25.00±2.94	14.53±0.49	8.38±0.19	0.0±0.0	<0.0001
FSC	4.68±1.52	2.02±0.66	0.59±0.13	0.0±0.0	<0.0001
MSC	9.02±1.99	3.64±1.15	0.94±0.49	0.0±0.0	<0.0001
SMI	48.14±7.93	43.02±2.41	27.40±2.76	0.0±0.0	<0.0001
Semen Volume	2.85±0.19	2.26±0.27	1.75±0.33	1.86±0.32	0.29

Key: TSC=Total sperm concentration, FSC=Functional sperm concentration, MSC=Motile sperm concentration, SMI=Sperm motility index and S.E.M=Standard Error of Mean, p<0.05=significant statistically, p>0.05=Not significant statistically, S=Significant, NS=Not Significant.

Table 5: Correlation between semen functional characteristics and DFI among infertile subjects

Parameters	R-value	P-value
TSC vs Big Halo	0.12	0.43
TSC vs DFI	-0.76	0.001
FSC vs Big Halo	0.12	0.07
FSC vs DFI	-0.53	0.002
MSC vs Big Halo	0.16	0.76
MSC vs DFI	-0.14	0.34
SMI vs Big Halo	0.21	0.09
SMI vs DFI	-0.58	0.001

Key: TSC=Total sperm concentration, FSC=Functional sperm concentration, MSC=motile sperm concentration, SMI=sperm motility index, DFI=Defragmentation index, p<0.05=significant statistically, p>0.05=Not significant statistically.

Table 6: Correlation between sperm functional characteristics and fertile control subjects

Parameters	R-value	P-value
TSC vs Big Halo	0.65	0.001
TSC vs DFI	-0.26	0.24
FSC vs Big Halo	0.21	0.34
FSC vs DFI	-0.23	0.06
MSC vs Big Halo	0.18	0.32
MSC vs DFI	-0.35	0.01
SMI vs Big Halo	0.64	0.004
SMI vs DFI	-0.25	0.08

Key: TSC=Total sperm concentration, FSC=Functional sperm concentration, MSC=motile sperm concentration, SMI=sperm motility index, DFI=Defragmentation index, p<0.05=significant statistically, p>0.05=Not significant statistically.

Fig 3a: Age distribution of study participants without sperm DNA fragmentation index

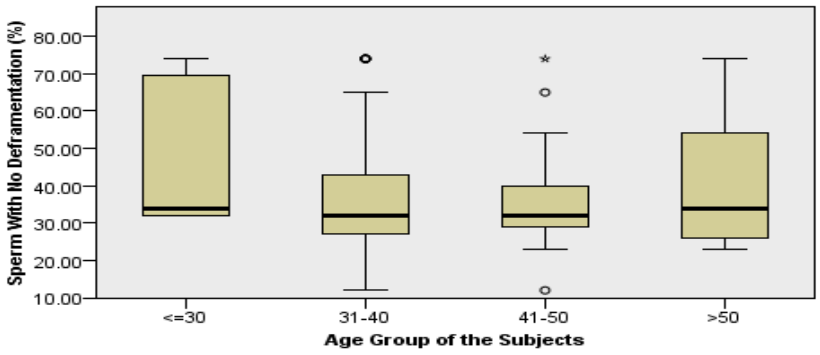
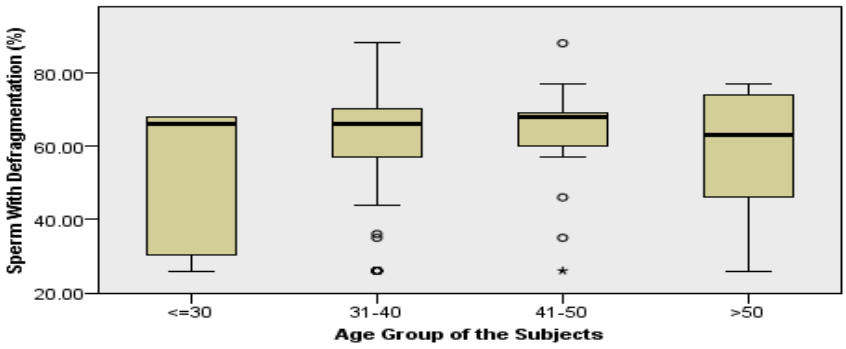


Fig 3b: Age distribution of infertile subjects with high sperm DNA fragmentation index



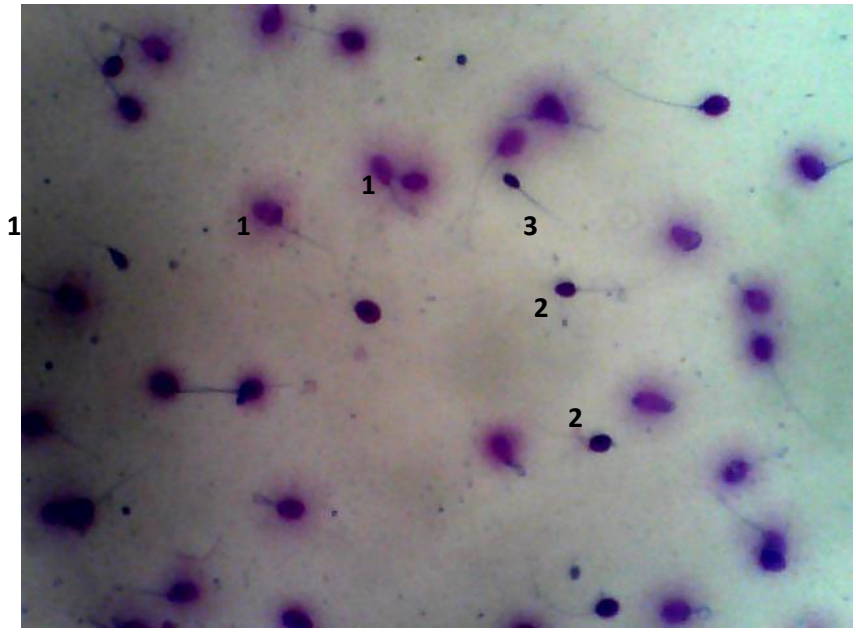


Photo 1: Photomicrograph of DNA damage using Halosperm DNA Fragmentation Kit
Keys:1=Big Halo Spermatozoa, 2=Medium Halo Spermatozoa, 3=Small Halo Spermatozoa and 4=Degraded Spermatozoa

(n=85, 68.0%) of the fertile patients were between 35-40 years of age. The educational status of the infertile subjects showed that 140 (56.0%) and 18 4(63.0%) of fertile and infertile men had tertiary education, equally, 146 (58.0%) and 178 (61.0%) of fertile and infertile men worked in the Civil service. Exactly 226 (90.0%) and 246 (84.0%) of the fertile and infertile men reside in the urban sector. Most of the respondents practice monogamy (fertile men 236 (94.0%) and infertile men 268 (91.0%)) respectively (table 1). Of the 280 infertile men, 124 (44.3%) were normospermia, 78 (27.9%) were mild oligozoospermia, 48 (17.1%) were severe oligozoospermia and 30 (10.7%) were azoospermia (Fig 1).

Table 2 shows the sperm indices of the infertile men, of the 124 (42.3%) normozoospermia, 84 (67.7%), 96 (77.4%) and 106(85.5%) had normal motility, normal morphology, and were viable, 50 (64.1%), 36 (46.2%) and 60 (76.9%) mild oligozoospermia were normally motile, normal morphology and viable, while 32 (66.7%), 22 (45.8%) and 36 (75.0%) with severe oligozoospermia were progressively motile, had normal morphology and viable.

Sperm DNA fragmentation in infertile men and fertile men (controls)

The sperm DNA fragmentation analysis was only done on 250 out of the 294 infertile men because 44 subjects were azoospermic (no sperm cells).

Figure 2 shows the percentage SDFI in both infertile men and fertile control subjects. The SDFI in infertile men 66.4±2.6% was significantly higher ($p<0.001$) than 16.0±1.1% in fertile control subjects. Exactly 100 semen specimens from the fertile control subjects were randomly selected and evaluated for SDFI, and 84 (84%) of the fertile men (controls) showed big halos (indication of no DNA damage) while 84 (33.6%) of the infertile men showed no DNA damage.

Table 3 shows the percentage distribution of sperm DNA fragmentation index in the study participants. The SDFI in infertile subjects with normozoospermia was 44.2±2.7%, mild oligozoospermia 65.4±2.4% and severe oligozoospermia 60.2±2.4% respectively.

The comparison of total sperm concentration (TSC), functional sperm concentration (FSC), and sperm motility index show significant difference ($p<0.001$) among normozoospermic, mild oligozoospermic and severe oligozoospermic infertile males. The semen volume among the subjects within the categories was not significantly different (Table 4).

Correlation Between sperm characteristics and sperm DNA Fragmentation index

Table 5 shows the correlation between SDFI and the sperm characteristic in the infertile men. It indicates that SDFI is inversely correlated with total sperm concentration (TSC)($r=-0.76$, $p<0.001$), functional sperm concentration (FSC) ($r=-0.53$, $p<0.002$) and sperm motility index (SMI) ($r=-0.58$, $p<0.001$), but an insignificant correlation was observed between SDFI and motile sperm concentration (MSC) ($r=-0.14$, $p>0.05$).

Conversely, among the fertile control subjects, there was a positive significant correlation between the Big halo (No DNA damage) and TSC ($r=0.64$, $p<0.001$) and SMI ($r=0.64$, $p<0.001$), a negative but significant correlation between DNA fragmentation index (DNA damage) and MSC ($r=-0.35$, $p<0.01$) but insignificant correlation between Big halo and FSC ($r=0.21$, $p>0.05$) was observed (table 6).

Figure 3b shows the percentage of semen samples with high SDFI was higher with increasing age, while the percentage of semen samples without sperm DNA fragmentation index decreased with increasing age of infertile male subjects.

Discussion

Successful embryonic development depends on the fertilization of an oocyte by intact and genetically complete spermatozoa (Sakkas *et al.*, 2010; Aitken *et al.*, 2013). Fragmentation of sperm nuclear DNA occurs frequently as a result of several factors such as lifestyle, infections, oxidative stress, sperm apoptosis, etc. (Sakkas

et al., 2010; Aitken *et al.*, 2013). The need to determine the contribution of sperm DNA fragmentation to male factor infertility is imperative, even though sperm with minimal DNA damage still retains fertilizing potentials (Ahmadi and Ng, 1999). This present study seeks to evaluate the proportion of sperm DNA fragmentation index and its association with sperm functional characteristics and age of infertile males investigated for infertility in Abuja, Nigeria.

In this study, the mean SDFI in ejaculates from infertile men was significantly higher than in ejaculates from fertile subjects. The observed SDFI was lower than 76% reported among infertile men in Poland (Marchlewska *et al.*, 2016), but higher than 41.3% reported among Japanese infertile men (Komiya *et al.*, 2014). In an experimental study, it was reported that repeated poor IVF outcomes, non-surgical causes of embryo transfer failure, spontaneous abortion and other causes of pregnancy loss often occur in genetically modified mouse strains with normal sperm morphology, count and motility (Li and Lloyd, 2020). In our study,the percentage of SDFI observed among normozoospermic infertile men was lower than observed for mild oligozoospermia and severe oligozoospermia infertile men. Again, these were significantly higher than 16.3% observed among normozoospermia but infertile men by Fernandez *et al.*, (2005). Previous studies in humans also indicates that subjects with infertility had a higher mean level of SDFI than controls. The area under the receiver operating characteristics curve was 0.93 for 20% SDFI. The calculated threshold value to differentiate between fertile controls and infertile subjects was 20% (Sergerie *et al.*, 2005). Sperm DNA fragmentation index threshold of 20% was reported to indicate the presence of infertility with high specificity and sensitivity (Santi *et al.*, 2018). Some researchers have suggested that the use of semen samples processed by density gradient centrifugation method when assessing SDFI may compromise the results, while others said that determination of SDFI in native or unprocessed semen may give higher specificity and positive predictive values for forecasting pregnancy outcomes following IVF (Simon *et al.*, 2011). Unprocessed ejaculated semen was used in this study.

A negative correlation was observed between SDFI and sperm functional characteristics in this study. This is consistent with previous studies in experimental animals (Li and Lloyd, 2020) and in humans (Komiya *et al.*, 2014). This is an indication that sperm DNA integrity as measured by SDFI may be highly affected in sperm with extreme low sperm count, poor motility and abnormal morphology. Since SDFI correlated negatively with TSC, FSC, MSC and SMI; which are vital for sperm to successfully fertilize oocytes, an inverse association between SDFI and fertilization rates in infertile men are expected. This may suggest the importance of evaluating the DNA integrity of spermatozoa either in the laboratory workup of infertile men; or for the purposes of fertilization in intrauterine insemination, in-vitro fertilization or ICSI, since sperm from infertile men contain high SDFI. The need to include SDFI as part of laboratory investigations of male infertility for the purposes of diagnosis, improving pregnancy outcomes in ART, predicting fertility and enhancing reproductive outcomes has been suggested (Li and Lloyd, 2020).

Sperm nuclear DNA plays a critical fundamental role in the fertilization and development of oocytes in animals and humans. Apart from the DNA strand breaks that are physiologically induced during spermatogenesis and spermiogenesis, other DNA damages do take place as a result of several exogenous or endogenous factors, thereby affecting DNA integrity during sperm maturation and storage in the epididymis (Moustafa *et al.*, 2004; Ramos *et al.*, 2004). Hence DNA integrity is often at risk and its evaluation may be a critical step in the assessment of sperm functional potential (Santi *et al.*, 2018).

The observed correlation between SDFI and TSC ($r= -0.76; P<0.001$), SDFI and FSC ($r= -0.53; P<0.002$) and SDFI and SMI ($r= -o.58; P<0.001$) is consistent with previous studies even though the earlier studies evaluated total motility, viability and morphology (Velez de la Calle *et al.*, 2008; Sivanarayana *et al.*, 2014; Santi *et al.*, 2018; Li and Lioyd, 2020). Findings of association between SDFI and conventional semen parameters have not been consistent. Whereas some authors reported a very strong inverse correlation between SDFI and sperm motility, morphology and density

(Velez de la Calle *et al.*, 2008), others observed a very weak inverse association between sperm concentration and SDFI, between normal morphology and SDFI (Zhang *et al.*, 2010). These authors also reported that the linear correlation between progressive motility and SDFI was moderate (Zhang *et al.*, 2010). Other authors reported that the proportion of degraded or fragmented spermatozoa in infertile normozoospermic males (11.1%) and infertile males with abnormal semen indices (12.2%) were not significantly different (Enciso *et al.*,2006). Furthermore, no correlation between SDFI and conventional sperm parameters has also been reported (Xie *et al.*, 2018).

The data from this study indicated that the percentage of semen samples with SDFI was higher with increasing age, while the percentage of semen samples without sperm DNA fragmentation index decreased with increasing age of infertile male subjects. This is an indication that senescence may contribute to increasing SDFI in spermatozoa. Senescence may contribute to higher SDFI in sperm cells via several mechanisms, including increased free radical generation and oxidative stress (Emokpae and Igharo, 2020). Excessive generation of free radicals more than the capacity of the available antioxidants can handle could negatively impact on lipids, causing lipid peroxidation of polyunsaturated fatty acid of membrane lipids. Oxidative damage to protein has a deleterious effect on protein function, nucleic acid oxidation, and DNA repair mechanism. These could result in nuclear DNA fragmentation, apoptosis and cell death. The generation of free radicals in excess of physiological needs is exacerbated by ageing among other causes. The senescent cells gradually increase in numbers in aging tissues and organs and have been shown to be a contributing factor to the generation of age-related phenotypes (Emokpae and Igharo, 2020). The exact mechanism responsible for the accumulation of senescent cells with aging that ultimately affects sperm DNA damage as men advance in age is not completely understood. Some have suggested that overexpression of antioxidant enzymes in experimental animals led to increased oxidative stress resistance, except mitochondrial catalase and thioredoxin, whose lifespan was not prolonged (Jang *et al.*, 2009; Perez *et al.*, 2011). There are indications that other mechanisms such as the P53 (a tumor suppressor molecule) which protects the cells from all anti-proliferative responses like the cell-cycle arrest, aging and apoptosis has been postulated.

Conclusion

The data presented in this study showed that the proportion of SDFI is higher in semen samples from infertile males than controls. The SDFI correlated negatively with sperm functional characteristics, and the proportion of SDFI increased with increasing age of study participants. It is suggested that the SDFI assay may be included in the routine investigation for the purposes of diagnosis of male infertility, forecasting fertility, enhancing ART outcomes and reproduction.

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