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Research Article

Oxidative Stress, DNA Fragmentation and Caspase-3 Regulation in HIV-1 Positive Men: A Study of Sperm Preparation

Margaretha Napitupulu [1], Friska Zai [1], Silvia W Lestari [2,*], Latisha Witjaksono [3], Zulfikar Drakel [3], Dhriti Bhavnani [3], Evy Yunihastuti [4], Gita Pratama [5], Ria Kodariah [6], Kusmardi Kusmardi [6]

[1]*Master's Programme in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta 10430 Indonesia*
[2]*Department of Medical Biology, Faculty of Medicine, Universitas Indonesia, Jakarta 10430 Indonesia*
[3]*Undergraduate Program in Medical Science, Faculty of Medicine, Universitas Indonesia, Jakarta 10430 Indonesia*
[4]*Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta 10430 Indonesia*
[5]*Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Indonesia, Jakarta 10430 Indonesia*
[6]*Department of Anatomic Pathology, Faculty of Medicine, Universitas Indonesia, Jakarta 10430 Indonesia*
[*]*Corresponding author: silvia.lestari@ui.ac.id; Tel.: +622131930379; Fax: +622131907281*

Abstract: The impact of human immunodeficiency virus (HIV) infection on male fertility, specifically sperm quality, remains inconclusive in global studies. However, stable HIV-positive men undergoing antiretroviral therapy (ARV) experience improved quality of life and increased life expectancy, which fuels their desire for parenthood. This study aimed to investigate sperm quality in HIV-positive men in Indonesia by examining oxidative stress, DNA fragmentation, and caspase-3 apoptosis after sperm preparation. A comparison was made between HIV-positive men (case group) and HIV-negative men (control group). Out of the initial 47 participants, seven were excluded due to conditions such as azoospermia, low motility, or high sperm count. The final sample comprised 40 participants, and semen samples were collected at the HIV Integrated Clinic in Jakarta, Indonesia. Sperm preparation was conducted using the Gradient Density Centrifugation method. The variable measured were the level of malondialdehyde (MDA) by Thiobarbituric Reactive Substances Assay (TBARS), DNA Fragmentation Index (DFI) by Sperm Chromatin Dispersion (SCD) method, and apoptotic rate represented as caspase-3 expression by immunocytochemistry (ICC). Those examinations were conducted before and after sperm preparation. Significant increases were observed in MDA levels, DNA fragmentation index (DFI), and caspase-3 expression in the case group before ($p < 0.01$) and after preparation ($p < 0.01$). These findings suggest that sperm preparation can selectively choose sperm based on oxidative stress, DNA fragmentation, and caspase-3 expression in HIV-positive patients. In conclusion, our study sheds light on sperm quality in HIV-positive men and underscores the potential of sperm preparation in improving fertility outcomes. Further research is necessary to explore management strategies and mechanisms for enhancing sperm quality in stable HIV-positive men receiving ARV treatment.

Keywords: Antiretroviral; Caspase-3; HIV; Oxidative stress; Sperm DNA fragmentation

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1. Introduction

In Indonesia, HIV infections occur either in remote areas or big cities, and this infection will develop into AIDS, which is susceptible to various opportunistic infections (Wahyuningsih et al., 2021). In the three decades, the recommended treatment for human immunodeficiency virus (HIV) infection by the National Institutes of Health (NIH) has been antiretrovirals (ARVs) that act on a wide range of HIV enzyme targets. Currently, there are three combination ARV therapies, including Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), and Protease Inhibitors (PIs) (Riono and Challacombe, 2020). The benefits of ARVs further increase life expectancy and desire to reproduce among HIV-positive men, especially in HIV serodiscordant couples. However, ARV therapy has been reported to harm male fertility due to oxidative stress on sperm (Dutta et al., 2019; Ivanov et al., 2016; Sharma, 2014). The composition of the plasma membrane, which is rich in polyunsaturated fatty acids (PUFA) and limited antioxidant ability, makes sperm cells very susceptible to oxidative stress that causes lipid peroxidation (Sharma, 2014). Malondialdehyde (MDA) is the final byproduct of lipid peroxidation, and its levels show a positive correlation with the presence of ROS in the seminal plasma of infertile men (Vessey et al., 2014). There is also sperm DNA fragmentation induced by oxidative stress, which can cause male infertility, impaired embryo development, and recurrent miscarriage (Rex et al., 2017; Alvarez et al., 2002).

Semen analysis is a routine examination performed on male infertility. Based on the recommendation of the World Health Organization (WHO) 2010, conventional semen analysis is based on an assessment of the concentration, motility, morphology, and vitality of sperm (Pfeifer et al., 2015; Dulioust et al., 2002). However, this analysis may be insufficient because sperm parameters do not reflect the potential for male gametes to penetrate the oocyte. In recent years, much attention has been focused on the effect of apoptosis on sperm quality (Hichri et al., 2018; Aitken and Baker, 2013; Ricci et al., 2002). The phenomenon of apoptosis in testicular spermatogenesis is evidenced by the presence of apoptotic agents in sperm cells in the form of caspase-3, which is active as a marker of the end of apoptosis. Furthermore, there is a hypothesis that apoptotic signaling, indicated by the presence of caspase-3 in sperm cells, may negatively correlate with conventional in vitro fertilization (IVF) results, potentially hindering the fertilization process (Hichri et al., 2018).

Assisted Reproductive Technology (ART), such as Intrauterine Insemination (IUI) or IVF - Intracytoplasmic sperm injection (IVF-ICSI), is a treatment option when HIV-positive men desire to have offspring (Engelman and Cherepanov, 2012; Sauer, 2005). This study investigates the sperm quality of HIV-positive men in terms of oxidative stress, DNA fragmentation, and caspase-3. The primary objective of this study is to investigate the potential adverse effects of HIV infection and antiretroviral therapy (ARV) on male fertility, specifically focusing on sperm quality in terms of oxidative stress, DNA fragmentation, and caspase-3 levels.

2. Methods

The study protocol and ethical permit for this study were approved by the ethics committee of the Faculty of Medicine, Universitas Indonesia, with ethics certificate number 458/UN2.F1/PPM.00.02/2019 on July 7th, 2020.

2.1. Design and Sample Study

The study was experimental research to explore the impact of ARV therapy on sperm quality, focusing on DNA fragmentation, apoptotic activity, and levels of oxidative stress. The case group consisted of HIV-positive men who were stable under ARV therapy, while the control group was HIV-negative men.

2.2. Management of HIV Patients

The HIV test for the evaluation of ARV therapy was performed by viral load and clusters of differentiation 4 (CD4) analysis. Viral load was measured using the RT-PCR technique, which is a widely recognized method for amplifying a DNA or RNA strain. (Lischer et al., 2020). The amplification signal was detected in the instrument recommended by the manufacturers. CD4 was tested using flow cytometry (BD FACSCalibur, Biosciences, USA). Meanwhile, the HIV test for screening of the control sample was a rapid HIV test (Bioline, Indofarma, Indonesia). In addition, the regimen of ART therapy in the HIV-integrated clinic of Dr. Cipto Mangunkusumo Hospital Jakarta is tenofovir disoproxil/TDF (Kimia Farma, Indonesia), zidovudine/AZT (Kimia Frama, Indonesia), and lamivudine/3TC (Kimia Farma, Indonesia) for NRTI types; efavirenz /EFV (Kimia Farma, Indonesia) and nevirapine /NVP (Kimia Farma, Indonesia) for NNRTI types; and lopinavir/ritonavir (LPV/R) (Azista, India) for PI types.

2.3. Semen Collection and Analysis

Semen samples were obtained by masturbation and collected into sterile containers after at least 3–5 days of sexual abstinence. After the liquefaction process, the semen was assayed according to WHO 2010 guidelines, including sperm concentration, motility, morphology, viability, and membrane integrity according to the WHO guidelines as written by Pilatz et al. (2014). All procedures were performed in a Class II Biological Safety Cabinet (BSC) (Pilatz et al., 2014).

2.4. Sperm Preparation

Sperm preparation was performed using the Density Gradient Centrifugation (DGC) method with the sperm grade medium (Sil-Select Plus, Fertipro, Belgium). A total of 2 mL 45% gradient medium (upper layer) and 2 mL 90% gradient medium (lower layer) were placed at the bottom of the tube, then 1 mL semen was transferred to the surface of the medium. Furthermore, the tubes were centrifuged stratified at 300 x g three times (20, 10, and 8 min).

2.5. Oxidative Stress Measurement by MDA Level

Measurement of sperm MDA levels was performed using the thiobarbituric acid reactive substance (TBARS) test method. Tetramethoxypropane/TEP (Sigma-Aldrich, Argentina) was used as the standard solution. The calibration equation for the TBA spectrophotometric method was as follows: y = 0.1428x - 0.2013. The semen samples (containing approximately 5 x 10^6 sperm) were prepared by adding tris citric acid /TCA (Sigma-Aldrich, Argentina) reagent at a volume of 400 µL and centrifuged at 10,000 x g for 5 min. The supernatant was discarded, while the pellets were added with SDS buffer solution (Sigma-Aldrich, Argentina). Once the solution cooled to room temperature, the supernatant was transferred to a cuvette for further analysis. Absorption measurements were performed using a spectrophotometer (Genesys, Thermo Fisher Scientific, US) at a wavelength of 530 nm to quantify the desired results (Hardianingtyas et al. 2025).

2.6. Assessment of Sperm DNA Fragmentation

Assessment of sperm DNA fragmentation was performed using the SpermFunc® DNA kit (BRED Life Science Technology Inc, China). The observations were demonstrated using a light microscope with a 40x magnification of 500 sperms. (Olympus, Japan) The assessment was divided into 1) Unfragmented DNA (large and medium halo) and 2) Fragmented DNA (small halo, without halo and degraded sperm). The percentage of unfragmented and fragmented DNA was calculated as sperm DNA fragmentation index (DFI), which consists of three classifications, namely good (DFI is 0–15%), (2) moderate (DFI > 15 ≤30%) and (3) severe (DFI > 30%) (Fernandez et al., 2005).

2.7. Immunocytochemistry of Caspase-3

The concentration of the semen sample was adjusted to 1 × 10^5 sperms/mL. Furthermore, the samples were centrifuged with cytospin (Sigma Chemical Co., Madrid, Spain). The supernatant was removed, and the pellet was smeared on poly-L-lysine coated slides. The slides were incubated

with 3% buffered formalin solution at room temperature for 20 min and permeabilized with 0.1% Triton X-100. Detection of caspase-3 was observed under a light microscope (Leica DM75, Germany) with a 40x magnification of 100 sperms. The results were calculated as the percentage of caspase-3 expression (%) (Mundijo et al., 2022).

2.8. Statistical Analysis

Mann-Whitney and Wilcoxon tests were used to analyze MDA and apoptosis parameters, while paired sample t-tests and Wilcoxon tests were used to analyze the DFI parameter. The significance level was set at 5% ($p \leq 0.05$).

3. Results and Discussion

3.1. Characteristic Data

Data analysis was conducted on a total of 40 men, comprising both HIV-positive individuals (case group) and 15 HIV-negative men (control group). Table 1 demonstrates the general characteristics of HIV-positive and HIV-negative men (Table 1). Age and smoking were similar between the two groups, while the control group had no alcohol status history. Between 40 and 45 years old is the age range for male fertility to decline, which is indicated by a decrease in the quality of sperm (Harris et al., 2011). In this study, the mean age of the two study subjects was under 40 years old (38.6 ± 5.91 and 32 ± 4.21) or within a range of fertility levels that were good enough to reduce the risk of bias in the study results. In addition, the contribution of paternal smoking status was reported to impact semen analysis parameters negatively, although other studies have not found this effect. In some cases, it even had a positive effect on sperm motility, while in other cases, there was an increase in sperm DNA nucleus damage (Harlev et al., 2015). Active smokers in both groups had the same percentage (28.6% *vs.* 27%), so that the subject's health condition was similar. A previous report by La Vignera et al. (2013) suggested that alcohol consumption did not significantly affect male fertility related to sperm parameters. This lack of significant impact could be attributed to various other factors that influence male fertility, such as genetic history and other individual-specific conditions (La Vignera et al., 2013).

Table 1 Characteristics of HIV-positive (cases) and HIV-negative (controls) men. HIV, human immunodeficiency virus; ARV, antiretroviral therapy; NRTIs, Nucleoside Reverse Transcription Inhibitors; NNRTIs, Non-Nucleoside Reverse Transcriptase Inhibitors; PIs, Protease Inhibitors; VL, Viral Load

Characteristics	HIV-positive (case)		HIV-negative (control)	
	Total	%	Total	%
Age (years)	38.6	5.91	31.88	4.21
Smoking status	10	28.6	4	27
Alcohol status	3	8.6	0	0
CD4 (cell count/µL blood)*	598	231.5		
Viral load				
Undetectable (<40 cells)	33	94.3		
Detectable (>40 cells)**	2	5.7		
ARV				
NNRI + NNRTI	27	77.1		
NRTI + PI	8	22.9		

*normal values: 410 – 1,590; **VL values of 2 patients were 57 and 73

In the HIV-positive men, the average CD4 count was 598 cells/µL of blood, while the viral load was mainly undetectable by 94.3%. The viral load of the other two HIV-positive men is 57 and 73 cells/µL of blood. CD4 values < 200 cells/µL of blood correlated with the proportion of motile sperm counts, while CD4 values > 350 cells/µL of blood positively correlated with good sperm vitality and motility (Ratnam et al., 2018; Nicopoullos et al., 2011). In addition, HIV-positive men

with low CD4 counts or opportunistic diseases may have decreased semen quality. The average CD4 count in the case group was 598 ± 231.5 cells/μL of blood; in other words, the CD4 count would not affect the semen analysis quality. Le Messurier et al. (2018) stated that an undetectable viral load could be maintained by providing regular ARV therapy for at least six months (Le Messurier et al., 2018). In this study, it was found that 93.4% of the majority of viral load was undetectable. This finding confirms several outcomes, including semen analysis results, MDA levels, oxidative stress levels, and caspase-3 apoptotic activity, were not the impact of HIV but the impact of ARVs. In addition, the use of combination types of ARV was mostly NRTI + NNRTI (77.1%). Meanwhile,the control group showed negative results for the HIV test. NRTIs cause oxidative stress and modify mitochondrial DNA as well as impair sperm motility (Porter and Sutliff, 2012; Pavili et al., 2010; Bujan et al., 2007; Lewis et al., 2003). This supports the study that most of the male HIV-positive subjects used the NRTI + NNRTI combination (77.1%), putting them at risk of experiencing oxidative stress and mitochondrial toxicity, which leads to decreased sperm quality.

3.2. Sperm Analysis

The results of the seminal analysis revealed significant impairments in nearly all sperm parameters among HIV-positive men compared to HIV-negative men, both before and after sperm preparation (Table 2). However, a notable decrease was found in sperm concentration, progressive motility, and the vitality of sperm ($p<0.05$) (Table 2). This study confirms Bujan et al. (2007) finding, which demonstrated decreased seminal volume and sperm motility in HIV-positive men compared to the control group (Bujan et al., 2007).

Table 2 Semen analysis of the HIV-positive (case) and HIV-negative (control) men before and after sperm preparation. HIV, human immunodeficiency virus

Sperm parameter	HIV-positive (Case)		HIV-negative (control)		P-value
	Before preparation (mean ± SD)	After preparation (mean ± SD)	Before preparation (mean ± SD)	After preparation (mean ± SD)	
Sperm concentration (million/mL)	32.38 ± 22.7	31.51 ± 24.63	63.19 ± 31.41	78.29 ± 31.93	0.000*(a); 0.000*(b); 0.851(c); 0.000*(d)
Total motility [a+b] (%)	51.51 ± 22.03	69.69 ± 20.72	78.27 ± 13.67	92.80 ± 7.57	0.000*(a); 0.000*(b); 0.000*(c); 0.002*(d)
Progressive motility [a] (%)	36.94 ± 15.97	62.49 ± 19	67.60 ± 17.97	84.29 ± 18.3	0.000*(a); 0.000*(b); 0.000*<c); 0.001*(d)
Morphology (%)	2.86 ± 2.13	5.83 ± 3.6	4.67 ± 1.92	7 ± 2.2	0.002*(a); 0.007*(b); 0.000*(c); 0.001*(d)
Vitality (%)	44.46 ± 20.67	67.26 ± 21	73.07 ± 11.15	83.43 ±11.7	0.000*(a); 0.001*{b); 0.000*(c); 0.029*(d)

p: significancy level; p value < 0.05; *: significant; a: HIV-positive vs. HIV-negative at before preparation; b: HIV-positive vs. HIV-negative at after preparation; c: before vs. after preparation of HIV-positive; d: before vs. after preparation of HIV-negative

Moreover, the results of previous studies also reported various alterations in sperm parameters in HIV-positive men. Additionally, Pilatz et al. (2014) and Savasi et al. (2018) also discovered that all HIV-negative men under ARV therapy encountered a deterioration in sperm parameters, such as volume, concentration, progressive motility, and normal morphology, in accordance with the reference value by WHO 2010 (Savasi et al., 2018; Pilatz et al., 2014). There is no precise mechanism identified as the definitive cause of sperm concentration and motility deterioration. However, some studies have reported an interrelationship between increasing seminal leukocyte count and declining sperm motility, which has been associated with increased production of ROS (Alahmar, 2019; Adewoyin et al., 2017; Lackner et al., 2010). Furthermore, a plausible mechanism behind the unwanted effect of ARV therapy on sperm disorders has been explained by Carr and Cooper (2000) that ARVs are implied in various metabolic and endocrine dysfunctions that can affect the testicle functions, genital tract, and sperm (Carr and Cooper, 2000). Another hypothesis was that disruption of the energy or ATP production resulted from sperm mitochondrial toxicity leading to impairment of sperm motility (Pereira et al., 2017). In addition, the effect of cold shock on spermatozoa cells can lead to mortality and vitality defects, changes in the lipid components membrane of spermatozoa, and changes in permeability (Larasati et al., 2022).

In addition, another finding of this study showed that sperm qualities after preparation in the HIV-positive men were also significantly lower compared to the HIV-negative men ($p < 0.05$) (Table 2). The comparison of the sperm qualities in both groups was also significantly lower before than after preparation ($p < 0.01$). Previously study stated that the increase in motile sperm after preparation is directly proportional to sperm free from oxidative damage (Henkel and Schill, 2003).

3.3. Sperm MDA Levels

This study showed that MDA levels in the HIV-positive men group were significantly higher than in the control group (Figure 1). Before preparation, the mean MDA levels in the HIV-positive and HIV-negative men groups were 23.11 ± 6.9 vs. 3 ± 1.34 nmol / mL ($p < 0.01$). MDA levels in the sperm of HIV-positive men were higher than that of HIV-negative men. One literature suggested that HIV infection-induced leukocytospermia, namely increased counts of morphonuclear polymer (PMN) and macrophages in seminal plasma, which is associated with abnormalities of spermatogenesis, abnormal sperm, prostate dysfunction, and seminal vesicles of HIV patients who are responsible for increasing levels of extrinsic ROS in seminal fluid (Pavili et al., 2010; Bujan et al., 2007).

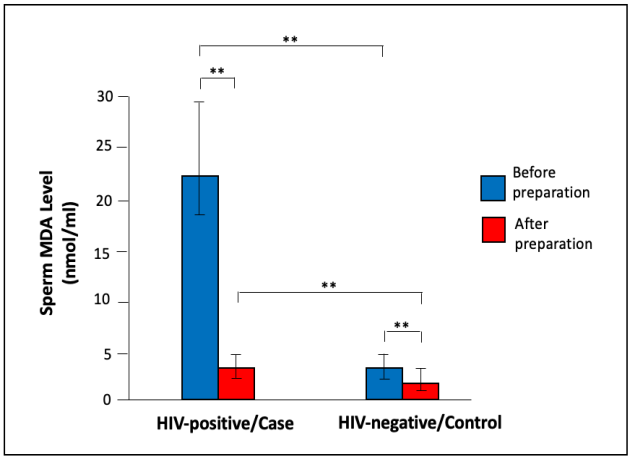

Figure 1 MDA levels of sperm in HIV-positive (case) and HIV-negative (control) men before and after preparation. Blue: before preparation, red: after preparation. ** = significant ($p < 0.01$). MDA, malondialdehyde; HIV, human immunodeficiency virus

Several studies have found a relationship between ARV therapy and mitochondrial toxicity, leading to decreased mitochondrial energy generation and changes in sperm metabolism

(Frapsauce et al., 2015; Kehl et al., 2011; Bujan et al., 2007; Moyle, 2004). ARV types such as tenofovir disoproxil, zidovudine, and stavudine have been shown to induce an oxidative stress response and impaired mitochondrial DNA integrity (Nagiah et al., 2015). Moreover, MDA levels were significantly higher in HIV-positive men after sperm preparation than in HIV-negative men ($p < 0.01$), and a previous study suggested that DNA fragmentation and apoptosis in sperms decrease after preparation, which is directly proportional to sperm being free from oxidative damage (Lestari et al., 2016).

3.4. Sperm DFI

DNA fragmentation index (DFI) was calculated by the number of halos formed on the sperm head. Halo is formed by the intact DNA strand coming out of the sperm head membrane. The more intact the sperm DNA, the larger halo is formed. Observation of sperm halos before preparation showed that small halo, no halo, and degraded sperm was predominantly found in the HIV-positive compared to HIV-negative men. Meanwhile, after sperm preparation, it was seen that large and medium halos dominated sperm halos in HIV-positive and HIV-negative men compared to before preparation (Figure 2A-D).

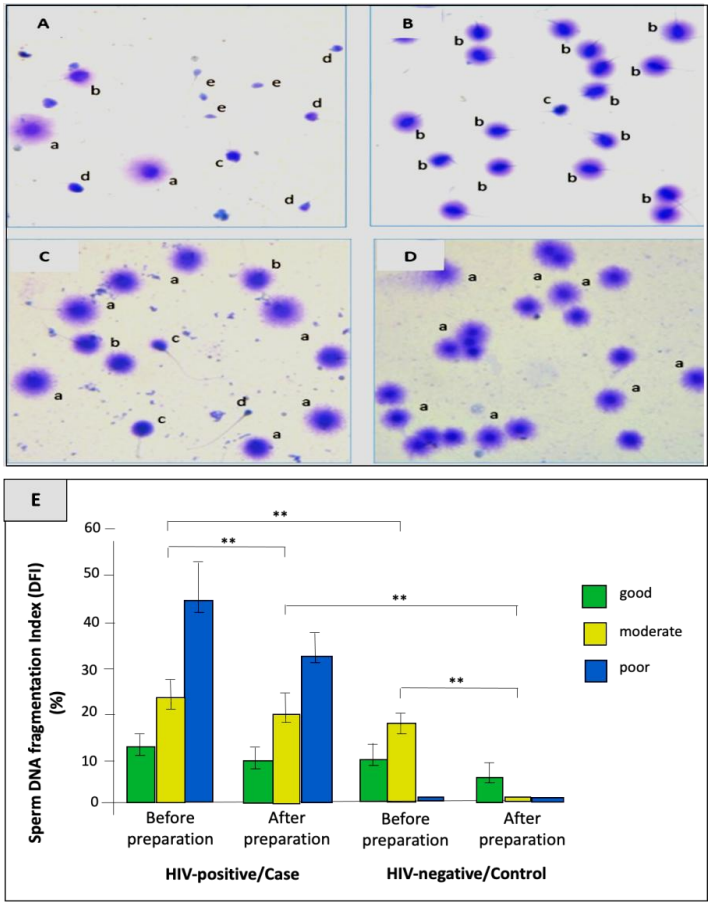

Figure 2 The sperm DNA fragmentation results. Sperm in HIV-positive men (A) before and (B) after preparation, sperm in HIV-negative men (C) before and (D) after preparation. (a) Indicates sperm with a large halo, (b) indicates sperm with a medium halo, (c) indicates sperm with a small halo, (d) indicates sperm without a halo, and (e) indicates degraded sperm. The observation was performed at 400x magnification. SCD, sperm chromatin dispersion; HIV, human immunodeficiency virus. (E) Sperm DNA fragmentation index (DFI) in HIV-positive (case) and HIV-negative (control) men, before and after preparation. Green: good category (DFI ≤ 15%), yellow: moderate category (DFI 15 – 30%), and blue: poor category (DFI ≥ 30%) ** = significant ($p < 0.01$). DFI, DNA fragmentation Index; HIV, human immunodeficiency virus

The study found that HIV-positive men had significantly higher sperm DFI than HIV-negative men. HIV-positive men with good sperm quality had an average DFI of 13.1%, while those with moderate and poor quality had DFIs of 24.1% and 42.8%, respectively. In contrast, HIV-negative men with good sperm quality had an average DFI of 10%, while those with moderate and poor quality had DFIs of 17% and zero, respectively. The results indicate that HIV-positive men had significantly lower sperm DFI than HIV-negative men ($p < 0.01$) (Figure 2E).

According to Takeshima et al. (2021), sperm DNA fragmentation is caused by internal factors such as oxidative stress, abortive apoptosis, and defects in the sperm maturation process. Takeshima et al. (2021) also found that DNA fragmentation can be caused by 8-OH-guanine and 8-OH-2'-deoxyguanosine products, with increased levels of 8-OHdG correlating significantly with DNA fragmentation and damage to DNA double chains. The use of antiretroviral drugs (ARVs) may also contribute to sperm DNA fragmentation through active cellular mechanisms, which can affect spermatogenesis and the functionality of sperm (Viswambharan and Murugan, 2021; Akhigbe et al. 2021; Fisher, 2016; Bart et al., 2002). Savasi et al. (2018) reported a significant increase in severe DFI in HIV-positive men receiving ARVs compared to those who did not receive ARVs ($p < 0.05$).

After sperm preparation, DFI in HIV-positive men with good sperm quality decreased to an average of 10.6%, while those with moderate and poor quality had DFIs of 19.4% and 31.7%, respectively. In contrast, HIV-negative men with good sperm quality had an average DFI of 7.7%, while those with moderate and poor quality had DFIs of zero. These findings show a significant decrease in DFI after sperm preparation in both HIV-positive and HIV-negative men ($p < 0.01$) (Figure 3). Similar results were reported by Viswambharan and Murugan (2021) and Lestari et al. (2016), who found a significant repair of sperm DNA integrity after sperm preparation compared to before preparation ($p < 0.05$). Sperm preparation eliminates sources of oxidative stress such as ROS and selects motile sperm while separating immature sperm, leukocytes, bacteria, and debris, which are toxic sources of ROS, making it an important step in ART (Takeshima et al., 2018; Engelman and Cherepanov, 2012; Grunewald et al., 2009).

3.5. Sperm Caspase-3 Expression

This study implemented an immunocytochemical method to identify caspase-3 expression in sperm cell samples from both groups. Our findings suggested an increased expression of caspase-3 in the sperm of the HIV-positive (39.36 ± 12.83 %) compared to the HIV-negative men (19.91 ± 7.61%) (Figure 3F). Apoptosis through multicellular cell death regulatory and signaling pathways is a physiological cell death program caused by DNA fragmentation. Moreover, caspase is the primary effector and transducer of apoptotic signaling, leading to programmed cell death. ROS that induces double-chain DNA damage can produce apoptosis (Takeshima et al., 2021). The apoptotic cascade in sperm can be caused by oxidative stress conditions that interfere with spermiogenesis, resulting in impaired remodeling of sperm chromatin formation, which is the substitution of structure from histones to protamine (Takeshima et al., 2021; Hichri et al., 2018).

Before sperm preparation, HIV-positive men showed higher caspase-3 expression in the midpiece (23.82 ± 18.84%) than in the acrosome region (16.2 ± 11.81%), while HIV-negative men had higher expression at the proximal part of the acrosome (15.82 ± 6.62%) than in the midpiece region (3.18 ± 2.89%) (Figure 4). Damage to sperm can activate caspase-3, leading to decreased motility, DNA fragmentation, and cell death. Previous studies have explored caspase-3 expression in sperm but not in HIV-positive men. Our study found that HIV-positive men had a higher distribution of caspase-3 expression in the acrosome and midpiece areas, mainly located in the acrosome region. Furthermore, after sperm preparation, caspase-3 expression was higher in HIV-positive men than in HIV-negative men but decreased in both groups. Our findings support the idea that sperm preparation can remove immature sperm and select motile sperm with less oxidative DNA damage. Grunewald et al. (2009) reported a relationship between damage to the

sperm midpiece and caspase-3 activation, and other studies have found a positive correlation between DNA fragmentation and caspase-3 activation in sperm (Grunewald et al., 2009).

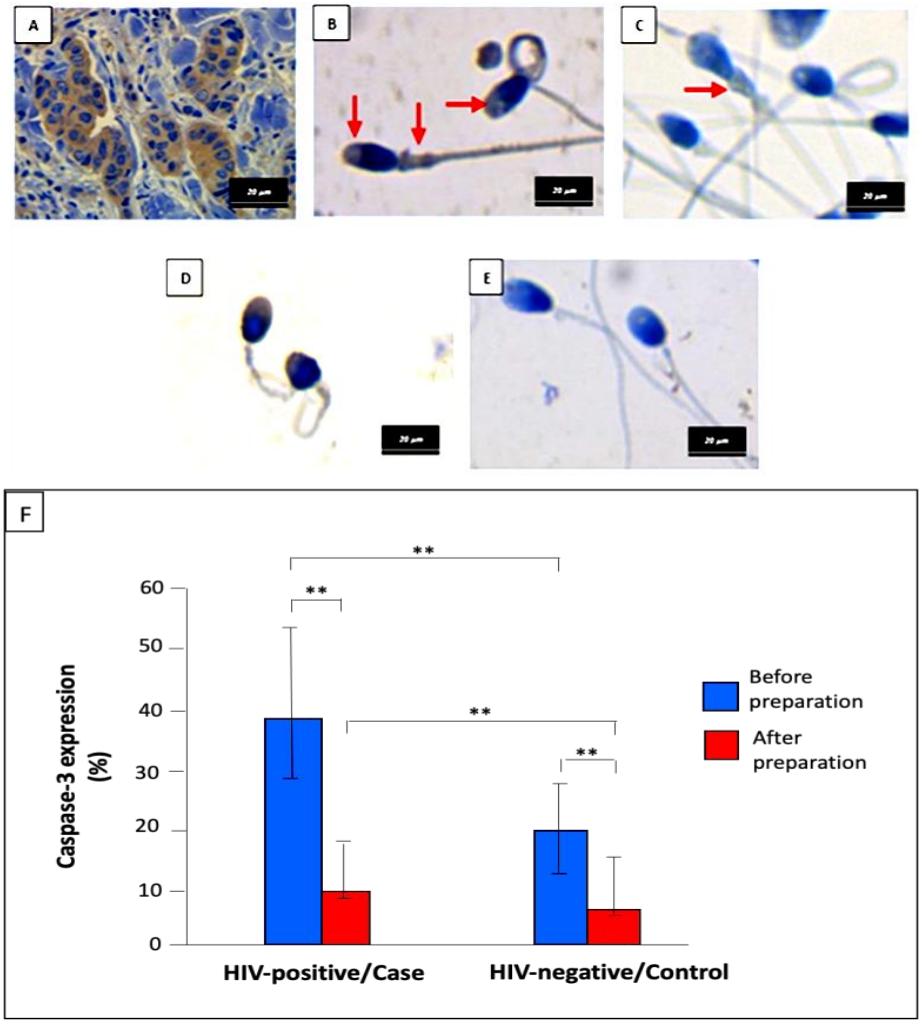

Figure 3 Immunocytochemical analysis of caspase-3 in sperm. (A) Breast cancer cells (as positive control), (B-C) Caspase-3 was expressed in the acrosome and midpiece of sperm in the HIV-positive men (case) and (D) in the acrosome in the HIV-negative men (control), and (E) Unexpressed caspase-3 sperm (as negative control). The red arrow indicates caspase-3 expression detected by DAB stain in ICC (brown color). Observation was performed at 400x magnification. HIV, human immunodeficiency virus. (F) Caspase-3 expression in sperm from seropositive and seronegative HIV-infected men was evaluated before and after preparation. Blue: before preparation, red: after preparation. ** = significant ($p<0.01$)

This study found that after sperm preparation, HIV-positive men had higher caspase-3 expression than HIV-negative men, but both groups had decreased caspase-3 expression. The study also suggests that sperm preparation can effectively select motile sperm by eliminating those with oxidative DNA damage and immature sperm, which may explain the observed depletion of caspase-3 positive sperm, as reported in previous studies (Lestari et al., 2018; Takeshima et al., 2017; Grunewald et al., 2009).

In addition to the previous information, it is important to note that while sperm preparation is a valuable method for selecting sperm based on various factors such as oxidative stress, DNA fragmentation, and caspase-3 expression, its application in infertility therapy for HIV-positive men holds significant potential. Furthermore, the assessment of MDA levels and DNA fragmentation can serve as useful tools for infertility screening. However, to enhance our understanding and

develop comprehensive management strategies for improving sperm quality in stable HIV-positive men receiving antiretroviral (ARV) treatment, further research is necessary. Exploring additional mechanisms and implementing novel management approaches will contribute to the advancement of infertility care in this specific population.

4. Conclusions

Sperm preparation is a useful method for selecting sperm based on oxidative stress, DNA fragmentation, and caspase-3 expression, which is demonstrated in this study by decreased value of each parameter after sperm preparation. Therefore, this procedure can aid in infertility therapy for HIV-positive men. MDA levels and DNA fragmentation could be useful for infertility screening. However, further research, such as in pharmacology, is needed to explore mechanisms of ARV therapeutic drugs in the male reproductive tract to support finding management strategies to maintain and improve sperm quality and to determine the safe use of post-wash sperm by assessing reproductive outcomes in stable HIV-positive men under ARV treatment.

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Author Contributions

First author of the manuscript, experimental design, data collection and analysis of semen quality: MN; methodology performance, data collection and analysis of sperm DNA fragmentation: FZ; supervisory role, funding acquisition, data curation, manuscript review and editing: SWL; data collection and analysis of MDA concentration: LW, ZD, DB; subject collector, manuscript review: EY; data collection and analysis of sperm caspase-3 expression, manuscript review: GP, RK, KK.

Conflict of Interest

The authors declare no conflicts of interest.

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