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The Effect of Sperm DNA Fragmentation on Fertilization and Embryogenesis Rates

Batara Imanuel Sirait
Department of Obstetrics and Gynecology, Medical Faculty, Universitas Kristen Indonesia
Corresponding Email: batara.sirait@uki.ac.id

***Abstract*:**
This research is the effects of sperm DNA fragmentation on fertilization and embryogenesis rates. The purpose of doing this research is to find out the effects of sperm DNA fragmentation on fertilization and embryogenesis rates. It is a quantitative study with cross-sectional design and carried out by taking male semen samples from infertile couples who participated in the IVF-ICSI program, examined WHO sperm analysis, and assessed the sperm DNA fragmentation index. For the analysis of the mean difference of 2 categories using independent sample t-tests while those of more than 2 categories use Anova. Analysis of categorical variable types uses the chi-square test. The result of this research shows that In the 10-month study period, the number of study subjects who met the inclusion criteria was 29 infertile couples with 29 ejaculate sperm samples and 200 oocytes. From the analysis of sperm DNA fragmentation index with fertilization rates, it appears that good fertilization (Z1 and Z2) is more common in groups with sperm DNA fragmentation index ≤30%, and statistically significantly different with $p < 0.05$, and from analysis of sperm DNA fragmentation index with embryo scoring seen better embryo scoring (grades A and B) in the sperm DNA fragmentation index group ≤30%, which was not statistically significant. It is concluded that the sperm DNA fragmentation rates can show a negative relationship with fertilization rates but can not show a statistically significant relationship with embryogenesis rates. A statistically significant relationship was seen between the World Health Organization (WHO) sperm analysis parameters and DNA fragmentation index examined using the SCD method.
Keywords:Sperm DNA fragmentation index, Fertilization, Embryogenesis.

1. INTRODUCTION

Infertility can be caused by both female and male or both factors. It is a complex situation, where often a combination of several factors plays an important role. Traditionally genital infections, endocrine disorders, and immunological factors are considered as the most common causes of male subfertility. However, at this time often genetic/molecular causes are found as contributing factors, such as chromatin damage which is considered as defragmentation of sperm DNA. However, 60-75% of cases of decreased semen quality cannot be explained and are therefore diagnosed as idiopathic infertility. The gold standard of diagnosis of male infertility or subfertility is sperm analysis which includes concentration, motility, and morphology according to standards determined by WHO. Over the past decade several sperm function tests have been recommended, including vital staining, hemizona assay, biochemical analysis of semen, anti-sperm antibody test, hypoosmotic swelling test, sperm penetration assay, reactive oxygen species (ROS)

tests, and computer-assisted sperm analysis (CASA) [1;2;3].

Several examination techniques have been proposed to study the disorder. Some that are still used today are the techniques of TUNEL, Comet, orange acridine, and sperm chromatin structure assay (SCSA). Furthermore, there is also an examination of Sperm Chromatin Dispersion (SCD) which turned out to be a fairly accurate examination to assess sperm DNA fragmentation. This examination is based on the presence or absence of halo by giving acidic liquid to sperm which is then followed by lysis buffer, halo DNA dispersion can be seen in the sperm nucleus with unfragmented DNA after the loss of core proteins and on the request with fragmented DNA, this halo looks small or not formed at all [4;5;6;7]. Our understanding of the female reproductive function and the importance of male-factors in infertility have increased significantly over the past decade. In the past, female partners were the main focus of attention and the husband factor was considered a relatively unusual cause of infertility. It is now known that abnormalities in men can be the sole cause of infertility in approximately 20% of infertile couples and play an important role in 20–40% of couples with reproductive disorders [8; 9; 10;11].

The effects of sperm DNA fragmentation on infertility have been the subject of several studies. Previously 10-20% sperm DNA fragmentation has been reported in ejaculate sperm. Infertile men with poor motility and morphology have been suspected to have increased sperm DNA fragmentation when compared to men with normal sperm parameters. Men with normal sperm parameters can also have high sperm DNA fragmentation, which can be an unexplained reason for infertility [12;13;14]. Aberrant chromatin packaging during spermatogenesis, defective apoptosis before ejaculation, or overproduction of reactive oxygen species (ROS) causes sperm DNA fragmentation, but the mechanism underlying the situation is unclear. There is still controversy over the effects of sperm DNA damage on reproductive output. Some examiners have found that clinical pregnancy is inversely affected by damage to sperm DNA in ICSI cases. Moreover, fertilization obtained through sperm which has DNA fragmentation can cause poor embryonic development, reduced implantation rates, and pregnancy. Another examiner found that sperm DNA damage caused ineffectiveness in fertilization, decreased embryo quality and pregnancy rates in IVF and ICSI [15; 16; 17; 18].

Many studies have shown that paternal effects can cause the repeated failure of assisted reproductive techniques. Many authors have shown that the paternal effect can be traced to the anomalous sperm chromatin organization. Sperm from subfertile men are characterized by being susceptible to acid-induced denaturation, reduced chromatin condensation, chromosomal anomalies, and/or increased DNA fragmentation. Many sperm seen with changes in the structure of chromatin harms the outcome of the TRB procedure. These studies focus on examining possible correlations between changes in paternal chromatin and fertilization rates, embryo division, blastocyst development, and clinical pregnancy in both IVF and ICSI [19;20]. These findings suggest that paternal genomic changes can jeopardize fertilization, embryonic quality as well as embryonic viability and continuity of pregnancy, leading to spontaneous abortion. At present, several studies in men and animals have underlined the importance of paternal factors, including male age or exposure to toxic material in cases of spontaneous abortion, but the relationship between early embryonic development after implantation in couples undergoing

TRB and examination of sperm DNA integrity is still a necessary further explanation. The effect of changes in sperm chromatin integrity on the early development of embryos after implantation is still debated [21; 22].

Mammalian fertility and subsequent embryonic development depend in part on the integrity of sperm DNA. It even seems that there is a threshold level of damage to sperm DNA (DNA fragmentation, abnormal chromatin, and protamine deficiency) wherein above that the rate of embryonic development and pregnancy can be disrupted. Sperm integrity testing has been developed and clinically applied. Sperm DNA fragmentation is increasingly understood as an important cause of infertility. Recent clinical research states that levels of sperm DNA fragmentation above 30% as measured by the SCSA method are incompatible with the initiation and occurrence of term pregnancy. Recent data shows that the value of the SCSA examination called the DNA Fragmentation Index correlates significantly with pregnancy rates both invivo and invitro. All pregnancies occur when the DNA Fragmentation Index number is less than 30% [23]. SCD (sperm chromatin dispersion) was not created as a substitute for SCSA. However, like SCSA, SCD can also distinguish sperm DNA fragmentation. The results of examining sperm DNA fragmentation by the SCD method are also consistent when compared with the results of examinations using the SCSA method. This shows that even with much simpler equipment and procedures an SCD examination is fast, relatively inexpensive, accurate, and can be repeated with an accuracy comparable to an SCSA examination. Therefore, SCD examination has the potential to be used as a routine screening test for sperm DNA fragmentation [24;25].

This result was confirmed by DNA breakage detection-fluorescence in situ hybridization (DBD-FISH), a procedure that uses restricted single-stranded DNA motifs resulting from DNA damage that can be detected and calculated. So DNA fragmentation that is reflected by the halo size can be determined accurately using SCD examination which is a simple, accurate, well-repeated, and inexpensive technique. In the SCD protocol, sperm nucleoids can be seen using a fluorosens microscope after staining with DNA specific fluorochrome (6-diamino-2-phenylindole [DAPI]) or with a light microscope after Diff-Quik staining. The ease of use and interpretation of SCD, as well as sensitive and reliable techniques, has the potential to be carried out in a variety of basic studies in clinical laboratories, in contrast to the more complex SCSA [26;27].

2. METHOD

This research is observational research, by taking semen samples of infertile men who participated in the IVF-ICSI program, examined WHO sperm analysis and assessment of sperm DNA fragmentation index. After ICSI, fertilization and embryogenesis were assessed. Then analyze by calculating the correlation. The study design was cross-sectional to see the correlation between the sperm DNA fragmentation index with the rate of fertilization and embryogenesis. Inclusion criteria were: Sperm of patients who participated in IVF using the ICSI method at Permata Hati Clinic Dr. Sardjito Yogyakarta who is willing to take part in the research. Exclusion Criteria: Couples who participated in IVF with the ICSI method at Permata Hati Clinic Dr. Sardjito Yogyakarta with her woman suffering from polycystic ovaries and or endometriosis. The sample size is Proportion

Sample, the patient who came to take the IVF program with the ICSI method at Permata Hati Clinic Dr. Sardjito Yogyakarta from July 2012 to May 2013. Researchers have obtained information on ethical eligibility (Ethical Clearance) from the Biomedical Research Ethics Commission in Humans, Faculty of Medicine, Gadjah Mada University Yogyakarta. Intact spermatozoa are dissolved in agarose matrix on the slide, given an acid solution for denaturation of DNA that contains damage, then lysis buffer is given to remove the membrane and protein. Agarose matrices make it possible to work without fixed sperm on an object-glass in an environment such as a suspension. The removal of the nucleus protein produces nuclei with central nuclei and peripheral halo from the dispersed DNA strands. By using fluorosens staining, the obtained sperm nucleus with increased DNA fragmentation produces a very small halo or absence of halo from DNA dispersion, where sperm with a low fragmentation level releases DNA strands that will form large halos.

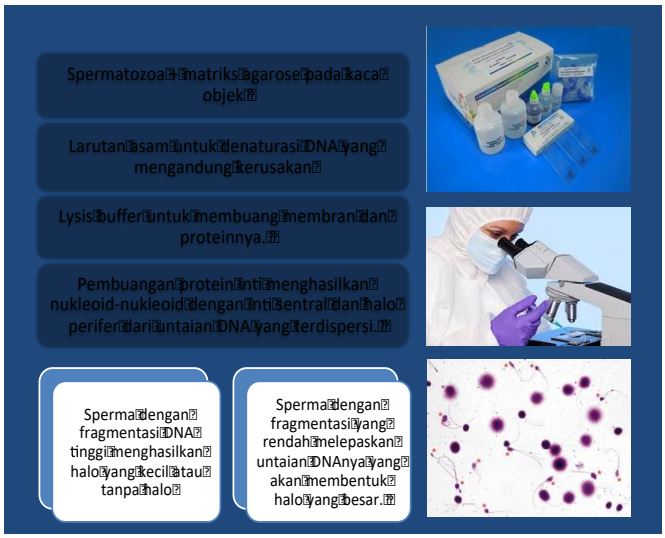

Figure 1. Flow Check of Sperm Chromatin Dispersio

The assessment of sperm DNA fragmentation examination results using the sperm chromatin dispersion method is done by looking at the size of the halo that forms around the nucleus of sperm cells.

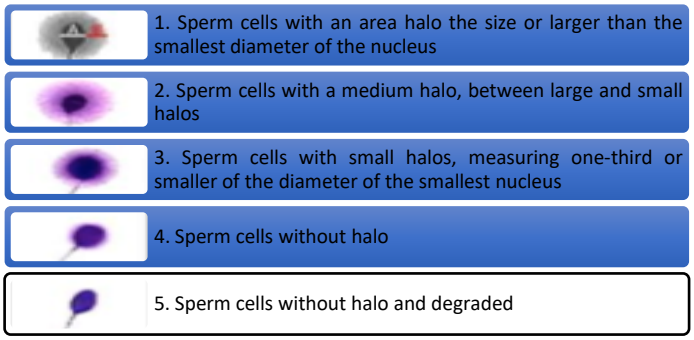

Figure 2. Assessment of Sperm DNA Fragmentation by SCD Method

Scott et al use the Z-scoring system to divide zygotes into four categories based on an assessment of the size and alignment of the nucleus and also the number and distribution of nucleoli [28].

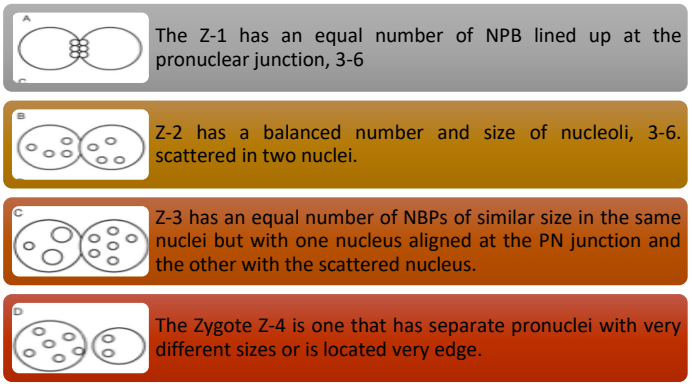

Figure 3. Z-scoring system [28]

Baczkowski T et al used a scoring system as below to divide embryos into four groups in which embryos included in groups A and B were considered good embryos, while those in groups C and D considered embryos that were considered to be unfavourable with the risk of developing congenital defects if an embryo transfer is done.

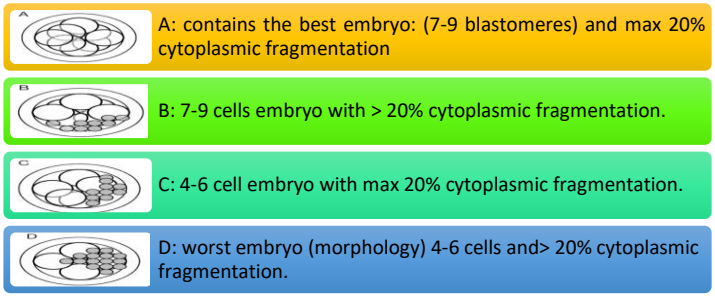

Figure 4. Morphological assessment of third-day embryos [28]

3. RESULT AND DISCUSSION

In our 10-month study period, the number of study subjects after excluding patients with a diagnosis of endometriosis and PCOS according to exclusion criteria was 29 infertile couples with 29 ejaculate sperm samples and 200 oocytes. The statistical test used is the parametric test. For the analysis of the mean difference of 2 categories using an independent sample t-test while those of more than 2 categories use ANOVA. Analysis of categorical variable types uses the chi-square test.

Table 1. Results of Analysis of Sperm DNA Fragmentation Index by examination ofWHO Sperm parameters

Variable	Fragmentation Index Sperm DNA	
	≤30% N (%)	>30% N (%)
WHO Sperm Analysis		
Normozoospermia	28 (23.1%)	0
Oligozoospermia	5 (4.1%)	11 (14.1%)
Asthenozoospermia	34 (28.1%)	20 (25.6%)
Oligoasthenozoospermia	3 (2.5%)	0
Teratozoospermia	20 (16.5%)	14 (17.9%)
Oligoasthenoteratozoospermia	31 (25.6%)	33 (42.3%)

***P*<0.00**

From Sperm DNA Fragmentation Index Analysis with WHO Sperm Analysis seen in sperm DNA fragmentation index group indeks30% is dominated by WHO sperm analysis results which are *asthenozoospermia, oligoasthenoteratozoospermia, normozoospermia, and teratozoospermia*. In groups>30%, it is dominated by *oligoasthenoteratozoospermia, asthenozoospermia, teratozoospermia*, and followed by *normozoospermia*. These results were statistically seen that there were significant differences p <0.05.

Table 2. Results of Sperm DNA Fragmentation Index Analysis with Fertilization Rates

Variable	Fertilization Figures				
	Negative	Z1	Z2	Z3	Z4
Sperm DNA Fragmentation Index					
≤30%	59 (60.2%)	9 (81.8%)	48 (60.8%)	1 (14.3%)	4 (100.0%)
>30%	39 (39.8%)	2 (18.2%)	31 (39.2%)	6 (85.7%)	0

***P*=0.027**

From the Analysis of Sperm DNA Fragmentation Index with Fertilization Figures, it appears that good fertilization (Z1 and Z2) is more in the group with sperm DNA fragmentation index ≤30%, but there are conflicting results where negative fertilization was also found more in the group with sperm DNA fragmentation index ≤30%. The results of the analysis of the table above between the DNA fragmentation index with the fertilization rate obtained statistically significantly different results p <0.05.

Table 3. Results of Analysis of Sperm DNA Fragmentation Index by Embryo Scoring

Variable	Skoring Embriogenesis				
	Negative	A	B	C	D
Sperm DNA Fragmentation Index					
≤30%	65 (59.1%)	7 (87.5%)	24 (55.8%)	24 (66.7%)	1 (50.0%)
>30%	45 (40.9%)	1 (12.5%)	19 (44.2%)	12 (33.3%)	1 (50.0%)

***P*=0.464**

Analysis of Sperm DNA Fragmentation Index with Embryo Scoring shows better embryo scoring (Grade A and B) in the sperm DNA fragmentation index group ≤30%, but there are conflicting results where negative embryogenesis is also found more in groups with index sperm DNA fragmentation ≤30%. The results of the above table analysis between DNA fragmentation index and Embryo Scoring obtained results that were not statistically significant p> 0.05.

Table 4. Analysis results of examining WHO Sperm parameters with Fertilization Rates

Variable	Fertilization				
	Negative	1	2	3	4
WHO Sperm Analysis					
Normozoospermia	8 (8.2%)	(36.4%)	6 (20.3%)	0	0
Oligozoospermia	5 (5.1%)	0	1 (13.9%)	0	0
Asthenozoospermia	27 (27.6%)	0	0 (25.3%)	(42.9%)	(100.0%)
Oligoasthenozoospermia	1 (1.0%)	(18.2%)	0	0	0
Teratozoospermia	19 (19.4%)	(18.2%)	3 (16.5%)	0	0
Oligoasthenoteratozoospermia	38 (38.8%)	(27.3%)	9 (24.1%)	(57.1%)	0

***P*<0.001**

From the Relationship between WHO Sperm Analysis with Fertilization Figures, there is better fertilization (Z1) found in the WHO sperm analysis group which is normozoospermia when compared with each of the other groups, followed by *oligoasthenoteratozoospermia, then subsequently oligoasthenozoospermia and teratozoospermia* with the respective numbers each the same. As for Z2, there are more in the asthenozoospermia group followed by aligoasthenoteratozoospermia, normozoospermia, teratozoospermia, and oligozoospermia. While the results of the most negative fertilization in the oligoasthenoteratozoospermia group. Results from the analysis of the above table between World Health Organization sperm analysis with fertilization rates obtained statistically significant results p <0.05.

Table 5. Analysis results of examining WHO sperm parameters by embryogenesis scoring

Variable	Scoring Embryogenesis				
	Negative	A	B	C	D
Analysis Sperma WHO					
Normozoospermia	1 (10.0%)	(62.5%)	(18.6%)	(11.1%)	0
Oligozoospermia	(4.5%)	0	4 (9.3%)	7 (19.4%)	0
Asthenozoospermia	8 (25.5%)	3 (37.5%)	1 (25.6%)	11 (30.6%)	1 (50.0%)
Oligoasthenozoospermia	1 (0.9%)	0	0	2 (5.6%)	0
Teratozoospermia	3 (20.9%)	0	8 (18.6%)	3 (8.3%)	0
Oligoasthenoteratozoospermia	2 (38.2%)	0	12 (27.9%)	9 (25.0%)	1 (50.0%)

***P*=0.006**

From the Relationship between WHO Sperm Analysis with Fertilization Figures, there is better fertilization (Z1) found in the WHO sperm analysis group which is normozoospermia when compared with each other group, followed by oligoasthenoteratozoospermia, then subsequently oligoasthenozoospermia and teratozoospermia with numbers from the other groups. WHO Sperm Analysis with Embryogenesis Scoring shows better embryo scoring (Grade A) in WHO normozoospermia sperm analysis when compared with each other group and also seen worse embryo scoring (Grades C and D) in the abnormal group namely asthenozoospermia and oligoasthenoteratozoospermia. Most negative embryogenesis scoring in the oligoasthenoteratozoospermia group was followed by asthenozoospermia and teratozoospermia. Results from the analysis of the above table Relationship between WHO Sperm Analysis with Embryogenesis Scoring obtained statistically significant results $p < 0.05$.

In this study, we tried to exclude factors that could affect the quality of oocytes such as PCOS and Endometriosis, so that the study subjects included in this study only consisted of cases of infertility caused by male factors, myoma uteri, both tubal non-patents, hydrosalpinges, and unexplained infertility. We did this to get more reliable results, although we therefore only got 29 infertile couples and 200 oocytes during the study period.

From the analysis of sperm DNA fragmentation index by examining WHO sperm parameters, it appears that the sperm DNA fragmentation index group ≤30% is dominated by an abnormal group but in the sperm DNA fragmentation index group> 30% we did not get normozoospermia whereas in the group sperm DNA fragmentation index ≤30% we get 23.1%. This is consistent with the study of Lin et al in his study with 86 cases of ICSI found there was an influence between sperm DNA fragmentation on sperm motility and also Irvine et al who stated that there was a very significant negative relationship between sperm concentration and sperm DNA damage [29;30].

From the analysis of sperm DNA fragmentation index with fertilization rates, it is seen that better fertilization rates are more numerous in the sperm DNA fragmentation index group ≤30%, according to the research of Muriel et al and Benchaib et al who stated a negative correlation between the level of sperm DNA

fragmentation with fertilization rates. [31;5;32]. However, the negative fertilization group was also more prevalent in the sperm DNA fragmentation index group ≤30%.

From the analysis of sperm DNA fragmentation index by the embryo, scoring can be obtained that better embryogenesis scoring occurs in the sperm DNA fragmentation index group ≤30%, by the results obtained by Bungum et al in his 2004 study in which biochemical pregnancy rates in the fragmentation group Sperm DNA <27% (51.2%) is higher than the sperm DNA fragmentation group> 27% (47.1%) and also Virro et al who stated that men with sperm DNA fragmentation> 30% had a higher risk for low blastocyst numbers (<30 %) and also the cessation of pregnancy [33;34;35]. However, we also found negative embryogenesis which was also higher in the sperm DNA fragmentation index group ≤30%.

From the results of an analysis of WHO sperm parameters examination with fertilization rates, it appears that the normozoospermia group produces the highest quality fertilization (Z1), but in the Z2 group it is dominated by abnormal groups, namely asthenozoospermia and oligoasthenoteratozoospermia. The most negative fertilization comes from the oligoasthenoteratozoospermia group. From the results of an analysis of WHO sperm parameters examination by embryogenesis scoring obtained the most Grade A embryogenesis score scoring in the normozoospermia WHO sperm analysis.

These variations in output from sperm DNA fragmentation may be explained as follows; First, sperm DNA fragmentation may not show similar damage in terms of quantity or quality. Second, the outcome may be a balance between sperm DNA damage and the ability to repair from oocytes [36;37;38;39]. Li Z et al in a systematic review and meta-analysis found that sperm DNA damage did not have a significant impact on the chance of getting a clinical pregnancy in patients undergoing IVF or ICSI [40;41;42;43].

There are conflicting results regarding the effect of sperm DNA fragmentation on semen parameters and also regarding the need for examination of sperm chromatin assays as routine laboratory examinations. Some studies report no relationship between sperm DNA fragmentation and semen parameters, while others find a negative relationship on some or all parameters.

Our data show significant results in the relationship between obtaining fertilization but not statistically significant in the relationship between sperm DNA fragmentation with embryogenesis even though there are significant differences between sperm DNA fragmentation groups ≤30% and> 30%. This might be due to the small number of research subjects in our study, but this is also made possible by the complexity of the genomic integrity of spermatozoa as a determining factor in TRB.

It is also well known that oocytes can repair fragmented sperm DNA, which is also likely to have a major influence on the outcome of the research. Besides sperm, DNA integrity cannot be considered an absolute picture of all the paternal effects that control early embryonic activity and development after ICSI/IVF [44].

4. CONCLUSION

In this study, it was seen that the sperm DNA fragmentation rate could show a negative relationship with the rate of fertilization but could not show a statistically significant relationship with the rate of embryogenesis. A statistically significant relationship was seen between WHO sperm analysis parameters and DNA fragmentation index examined using the SCD method. Based on this study it can be suggested that examination of sperm DNA fragmentation be included as an integrated part of routine sperm analysis examinations in infertile patients.

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