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Relationship between sperm DNA fragmentation and embryo development in IVF patients

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ABSTRACT

Infertility is a condition in married couples who have regular and adequate sexual intercourse for more than one year without using contraception, but have not had a pregnancy or offspring. Factors that cause infertility can be caused by men, women, or even both. Approximately 10% - 15% of couples of reproductive age experience infertility, with the contribution of the male factor, which is equal to 40% -50%. The analysis of embryo development and spermatozoa DNA fragmentation aims to analyze the function of spermatozoa in infertile men and to determine the relationship between sperm DNA fragmentation and embryo development in IVF patients. This study was an experimental study with a total of 30 samples of husband's patients who carried out the IVF program who met the researcher's inclusion criteria. The statistical test results obtained showed that there was a significant relationship between the DNA fragmentation index (DFI) ($P<0.05$), therefore it can be concluded that further research is needed so that many more things can be explored. of other causes of infertility.

Introduction

Infertility is a condition in married couples who have had sexual intercourse regularly and adequately for more than one year without using contraception, but have not obtained pregnancy or offspring [1]. Factors that cause infertility, It can be caused by men, women, or even both. Approximately 10% - 15% of couples of reproductive age experience infertility, with The contribution of the male factor, which is equal to 40% -50%.The male factors involved include genetic factors, age, infection, auto-antibodies, testosterone deficiency, hypogonadism, cancer, environmental factors, medication side effects, retrograde ejaculation, vasectomy, varicocele, and other unknown causes.

One way that can be used to determine the causes of infertility in men isby examining conventional semen analysis [2]. From the results of semen analysis in infertile men, the results can be obtained in the form of: asthenozoospermia (percentage of motile sperm less than 40%), oligozoospermia (sperm concentration less than 15 million/ml), teratozoospermia (normal sperm morphology less than 4%) or a combination of all three (oligoastenoteratozoospermia)[3,4]. Conventional semen analysis has been considered as the basis for laboratory tests for the initial diagnosis of infertile factors in men. If semen analysis is carried out with good quality and methods, then this examination can be used as an important reference for male fertility potential. The information obtained is based on the analysis parameters Conventional semen reflects the process of spermatogenesis to a certain stage which determines the functional competence and potential for sperm fertility.

It is known that sperm concentration, motility, and morphology are related to intracellular structure of sperm during spermatogenesis in the testis, maturation in the epididymis and normal plasma seminal moieties. Semen analysis may reveal extreme sperm dysfunction, such as azoospermia or globozoospermia which are known to negatively affect fertilization. However, even though it is considered capable of providing benefits in determining male infertility factors, conventional semen analysis still has limited diagnostic potential. In addition, about 15% of men with a normal semen analysis profile also experience infertility problems. Thus, a more accurate test application is needed to determine the functional etiology of male infertility.

From various studies that have been developed, it is known that sperm DNA integrity also influences the incidence of infertility. Abnormalities in the paternal genome characterized by DNA damage may indicate male subfertility. About eight percent of infertile men have abnormal DNA integrity even with normal semen analysis results. DNA fragmentation examination is an examination to assess the integrity of sperm DNA and the ability of sperm to fertilize an egg. In addition, abnormal sperm DNA integrity can also cause disturbances in embryo development, although it is not associated with poor fertilization rates [5].

Sperm DNA fragmentation can be measured and expressed as the Sperm DNA Fragmentation Index (IFD) by counting the number of sperm with fragmented DNA and compared to the total sperm count. Sperm with unfragmented DNA had large and medium halos, whereas sperm with fragmented DNA had small, non-halated or degraded halos. According to research by Silva, men with higher DNA fragmentation (small halo/no) are likely to experience infertility which is also higher. The IFD threshold for humans was first established using data from 200 couples of childbearing age suspected of trying to conceive naturally in the "Georgetown Male Infertility Factors Study". The data from this study were used to set statistical thresholds for IFD,

There are various techniques that can be used to measure the degree of DNA fragmentation of human sperm. The capacity of these human sperm DNA damage testing techniques to accurately predict sperm DNA damage depends on both technical and biological aspects. The sperm DNA fragmentation examination method that currently exists is the Sperm Chromatin Dispersion test(SCD), SCSA, Comet, TUNEL. In SCD, the basis of the examination lies in the different response by the sperm nucleus to fragmented DNA compared to intact DNA. Controlled denaturation of the DNA followed by extraction of nuclear proteins gives rise to partially deproteinized nucleoids, in which the DNA strands dilate, forming halos of dispersion of chromatin (halos), so that the fragmented sperm nucleotides form little or no halos [6].

Analysis of spermatozoa DNA damage can reveal fertility disorders in infertile men. Spermatozoa DNA damage can cause early embryonic death and abortion. Spermatozoa DNA damage greatly affects the quality of spermatozoa, fertilization rate, preimplantation development and embryo Development [7]. The level of spermatozoa DNA damage greatly influences embryo development and causes miscarriage [8] and spermatozoa DNA damage is negatively correlated with the level of pregnancy [9]. The higher the damage to the spermatozoa's DNA, the lower the pregnancy rate.

In a previous study, it was found that there was a miscarriage at the rate of damage to spermatozoa DNA of 37.11%, amounting to 14.31%. Vassilev also reported a miscarriage of 18.87% at the 45th day of gestation [8]. These results contradict the opinion of Rybar, which stated that the standard sperm DNA damage for cattle is 10% to 20% not recommended for fertilization while for humans it is more than 30% (Rybar et al., 2004). According to Evenson's research, the standard for spermatozoa DNA damage that is not recommended for fertilization in humans is 25-30%. Damage to spermatozoa DNA in humans if it exceeds 30-40% can cause miscarriage and it is not recommended to be used as frozen semen [6].

Method

Research conducted by researchers is direct observation. The data used is in the form of laboratory results from 30 couples who did the IVF program. Laboratory results taken were spermatozoa DNA fragmentation, and observations of patient fertilization and embryogenesis. The research was conducted at the Siloam Fertility Center and was carried out for 12 months, from November 2021 to November 2022. This period is the time taken by the author, starting from preparing the proposal to reporting the results of the research.

1. The target population in this study were couples who had an IVF program
2. The target population in this study are infertile Man
3. The affordable population in this study were infertile men who had their spermatozoa analyzed at the Siloam fertility center.
4. The sample of this study is an affordable population that fits the inclusion criteria and does not fit the exclusion criteria.

Inclusion Criteria

- The characteristics that must be met are married couples who carry out the IVF pregnancy program
- Female patient good prognosis
- First cycle
- The patient chooses the icsi method
- Embryo culture

Exclusion Criteria

- Azoospermia
- Oocytes not in the poor category (degeneration, abnormal, GV)

Results and Discussion

The subjects of this study were taken from patients who had undergone IVF (In Vitro Fertilization) pregnancy programs and analysis of sperm DNA fragmentation. The analysis of the characteristics of this study referred to the WHO laboratory manual for the examination and processing of human semen 2020. In this study, 30 samples were used and had various images as shown below

From Figure 1 it can be seen that the research subjects consisted of:

- DFI poor (Interpretation of results >30%)
- Moderate DFI (Result interpretation < 30%)
- DFI Good (Interpretation of results <15%)

The IFD value indicates the percentage of DNA fragmentation, a large IFD value indicates the amount of fragmented DNA. Of the selected subjects, the mean IFD value was

28..26. All subjects consisted of 12 subjects with bad IFD, 6 samples with moderate IFD and 7 samples with good IFD.

Results of Spermatozoa DNA Fragmentation Test

Spermatozoa DNA fragmentation test at the Siloam Fertility Center SHLV minimally invasive clinic using the SCD method. This examination will produce a halo image on the head of the spermatozoa. The resulting halo is then compared with the smallest diameter of the spermatozoa head. This comparison illustrates the fragmentation of spermatozoa DNA. Spermatozoa with large and medium halos showed spermatozoa DNA without fragmentation while spermatozoa with small, no halo and degraded spermatozoa showed fragmented spermatozoa DNA. The results of the spermatozoa DNA fragmentation test will produce an figure 1 below.

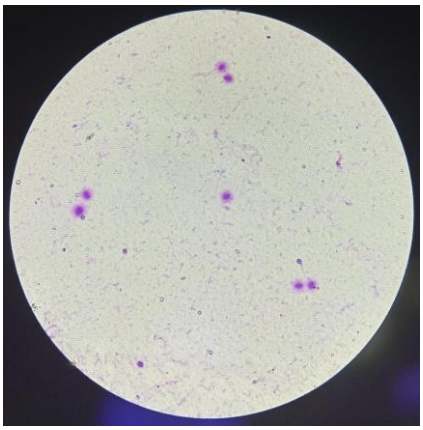

Figure 1. Spermatozoa DNA Fragmentation

The hypothesis "There is a relationship between fertilization, embryo development and sperm DNA fragmentation in infertile men" will be tested using the Pearson correlation test. Previously, a normality test was carried out for the distribution of data on both variables using the Kruskal Walls test, which obtained the p value of the DNA fragmentation index (= 0.91) and the value of the variable embryo development (p= 0.28). Spermatozoa DNA fragmentation and embryo development are said to be correlated if the correlation value ranges from 0 to 1 which can be positive or negative with a significant value <0.05.

In this study it can be concluded that there is a significant low correlation (p = 0.28) or even no correlation at all (p = 0.075). this can be interpreted that when the value of spermatozoa DNA fragmentation is high, the tendency of the results of embryo development is good or even vice versa. This can be seen in the image of the statistical test results

Table 1. Correlations

			Tipe_DFI	Embryo	Fertilisasi
Spearman's rho	Tipe_DFI	Correlation Coefficient	1000	.288	.075
		Sig. (2-tailed)		.163	.720
		N	25	25	25
	Embryo	Correlation Coefficient	.288	1.000	.019
		Sig. (2-tailed)	.163		.928
		N	25	25	25
	Fertilisasi	Correlation Coefficient	.075	.019	1.000
		Sig. (2-tailed)	.720	.928	
		N	25	25	25

Then the researchers tried to examine the differences in the proportion of spermatozoa DNA fragmentation groups in embryonic development. This was tested using the Mann-

Whitney Test. In vitro Fertilization (IVF) or the process of fertilization outside the body is a procedure developed to treat infertility or infertility problems that aim to produce a pregnancy. To date, more than 5 million babies have been born with IVF worldwide. The concept of fertilization outside the body dates back to 1890, but the first baby to be successfully born through this technique only appeared in 1978 in the United Kingdom which was conceptualized by Patrick Steptoe and Robert Edwards. In predicting the success of IVF, many things are associated with factors that increase or reduce pregnancy rates or IVF success rates. Factors associated with the success of IVF are age, ovarian condition, FSH levels, egg number, embryo development, oocyte status or picture at the time of sampling,

Factors that cause infertility can be caused by men, women, or even both. Approximately 10% - 15% of couples of reproductive age experience infertility, with the contribution of the male factor, which is equal to 40% -50%.The male factors involved include genetic factors, age, infection, auto-antibodies, testosterone deficiency, hypogonadism, cancer, environmental factors, medication side effects, retrograde ejaculation, vasectomy, varicocele, and other unknown causes [1]. One way that can be used to determine the causes of infertility in men isby examining conventional semen analysis. From the results of semen analysis in infertile men, results can be obtained in the form of: asthenozoospermia (percentage of motile sperm less than 40%), oligozoospermia (sperm concentration less than 15 million/ml), teratozoospermia (normal sperm morphology less than 4%) or a combination of all three (oligoastenoteratozoospermia) [3,4].

Conventional semen analysis has been considered the basis of laboratory tests for the early diagnosis of male factor infertility. If semen analysis is carried out using good methods and quality, this examination can be used as an important reference for male fertility. However, this conventional sperm examination only looks at the sperm from its morphology, not to see deeper sperm damage. Therefore it is advisable to do a sperm DNA fragmentation examination for couples who are planning to become pregnant. We need to know that examining sperm DNA fragmentation is very important to do, because sperm cells themselves are different from somatic cells, human spermatozoa have structural and functional integrity packaged in a unique system. Human spermatozoa DNA material is packaged with the help of special proteins that regulate the condensation and decompression processes through certain mechanisms. The balance of this process is synchronized by the organizational structure of the spermatozoa DNA. Structurally, most of the spermatozoa DNA is coiled into a toroid, a small part binds to histones and the rest is attached to the matrix of the spermatozoa core matrix-attachment regions (MARs). The most dense organization of spermatozoa DNA is within the toroid. During the maturation process, most of the histone proteins associated with DNA are replaced by protamine.

The interpretation of the results on reading sperm DNA fragmentation is divided into 3 categories: A. Poor if the results read sperm DNA fragmentation > 30%, B. Moderate if the results read DNA fragmentation < 30% and C. Good if the results read sperm DNA fragmentation <15%. Damage to spermatozoa DNA or chromatin sequence can occur at any stage during spermatogenesis. The positive relationship between poor spermatozoa parameters and DNA damage in mature spermatozoa points to problems of spermatogenesis in certain individuals. Damage to DNA fragmentation in sperm has two causes, namely internal factors and external factors. Internal factors include effects on the process of maturation of spermatozoa, oxidative stress and abortive apoptosis.

Spermatozoa have antioxidants and are limited in number according to the small volume of cytoplasm. These conditions make spermatozoa susceptible to oxidative stress caused by reactive oxygen species (ROS). In addition, the plasma membrane of spermatozoa which is rich in unsaturated fatty acids maintains membrane fluidity, causing spermatozoa to easily bind to ROS. This mechanism causes oxidative stress as a result of peroxidation of the plasma membrane, causing damage to spermatozoa and their defense mechanisms. The process of

packaging spermatozoa DNA is unique and has a complicated mechanism. Such complexity can expose DNA to damage that can occur at any stage.

In this research, the hypothesis does not match the results of the study because there is no correlation between poor DFI and embryonic development because in the IVF program we perform ICSI actions, where one sperm cell is injected in one egg, in ICSI action we can see and select sperm with the best morphology, because the sperm we choose is in a moving condition, while in DNA examination of sperm fragmentation, the sperm we see is still and colored so that if it is patient with the results of poor DFI analysis we will be more be careful in selecting sperm for us ICSI or mating with oocytes. So that fertilization and embryonic development are as we expect. As for patients with good fragmentation DNA analysis results, we can suggest doing conventional IUI or IVF procedures.

Conclusion

The conclusion of this study is that there is a significant negative correlation (p=0.0075) with (p=0.28). Based on these results and discussion, the researchers suggest using the spermatozoa DNA fragmentation index to assess the functional quality of spermatozoa more accurately without neglecting the value of embryo development tests

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