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Original Research Article

Negative correlation of Modified Sperm Stress Test with Leukocyte in Sperm but not with the sperm concentration and motility

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Abstract

Background: Sperm analysis is an initial detection procedure of male infertility recommended by the WHO. Sperm motility as one of the important parameters in sperm analysis has a close correlation with various determinants of male infertility and the success of Assisted Reproductive Technology (ART). Several laboratory assays were carried out as a complement to sperm analysis to describe the sperm ability for fertilization. One of the assays was the modified sperm stress test (MOST) to predict oocyte fertilization in In-Vitro Fertilization (IVF).

Objective: This study aimed to assess the correlation between MOST ratio and sperm parameters in infertile men.

Methods: This study is a descriptive-analytic study by taking sperm analysis and MOST data from 864 males with infertility problems who went to an infertility clinic, Sadewa IVF, Yogyakarta, Indonesia from May 2015 to November 2020.

Results: The mean sperm volume and concentration of the 864 patients were 3.04 mL and 35.74 x 10^6 / mL respectively. The mean forward motility and normal morphology were 47.93% and 2.07% respectively. The mean leukocyte cell number was 0.84%, and the mean MOST ratio was 0.51. The results showed a positive correlation between sperm concentration (r = 0.235) and motility (r = 0.302) with the MOST ratio. Meanwhile, the leukocyte parameter negatively correlated with the MOST ratio (r = -0.076) and no correlation between other sperm parameters to the MOST ratio.

Conclusion:

There is a positive correlation between sperm concentration and motility with the MOST ratio, and a negative correlation between the number of leukocytes and the MOST ratio. This result supports the findings of previous studies regarding the importance of sperm motility examination in the success of ART. To the best of our knowledge, this is the first study to assess the MOST in male infertility patients with the big sample size.

Keywords: *Male infertility; Sperm analysis; Fertilization; Modified sperm stress test*
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INTRODUCTION

Infertility is described as the inability to achieve a clinical pregnancy after 12 months in a sexually active, not using contraceptive methods, and occurs in 10-15% of couples.[1] Based on the recommendations of the AUA/ASRM 2021, evaluation of the causes of infertility must be carried out on male factors as well as female factors.[2] WHO compiles a standard sperm analysis consisting of several parameters, including volume, concentration, motility, and morphology.[3] Although this examination provides important information for the initial evaluation of men with infertility, a sperm analysis is not a fertility test.

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Table 1. Mean ± SD, minimum, and maximum values of sperm parameters and MOST ratio in infertile men

Value	Volume	Concentration	Motility	Morphology	Leukocyte	MOST
Mean ± SD	3.04 ± 1.2	35.74 ± 27.43	47.93 ± 16.45	2.07 ± 1.3	0.84 ± 1.08	0.51 ± 0.21
Minimum	0.80	3.50	4	0	0	0
Maximum	9.50	251.50	88	10	16.50	0.98

Table 2. Correlation between sperm parameters and MOST ratio in infertile men

Seminal Parameters		MOST Ratio		Correlation Coefficient	P
		<0.39	≥0.39		
Volume	<1.5mL	20	38	0.055	>0.05
	≥1.5mL	201	605		
Concentration	<15x10^6/mL	91	117	0.235**	<0.01
	≥15x10^6/mL	130	526		
Motility	<32%	84	73	0.302**	<0.01
	≥32%	137	570		
Morphology	<4%	203	568	0.050	>0.05
	≥4%	18	75		
Leukocyte	<1 x 10^6/mL	177	466	-0.076*	<0.05
	≥1 x 10^6/mL	44	177		

The results obtained cannot show the functional potential of spermatozoa to achieve a good maturation process for fertilization. This is unique because sperm analysis is a test related to fertility but cannot directly describe the fertilization function of the spermatozoa.[4] Several laboratory assays were carried out as a complement to sperm analysis assay to describe the ability of sperm to achieve fertilization, one of the assays was the modified sperm stress test (MOST). These laboratory techniques are based on the previous methods introduced by Alvarez et al.[5] The difference between the MOST and sperm stress test is the shorter incubation time and the intrinsic reaction mechanism.[6] A previous study showed that the modified sperm stress test (MOST) ratio was statistically significantly correlated with the fertilization rate in patients undergoing IVF. The threshold of the MOST ratio that was significant to the fertilization rate was 0.39. A MOST ratio of more than 0.39 is associated with better in vitro fertilization rates.[6] The basic principle of MOST is a sperm survival test to assess sperm motility which is known to be correlated to various factors that affect the success of ART, such as sperm DNA fragmentation and oxidative stress.[7] The results of sperm motility examination are one of the important points in deciding which insemination procedure can be performed. The Intra Uterine Insemination (IUI) procedure, required at least 5 million motile sperm produced from the ejaculate. It is recommended to conduct an IVF procedure if 2-5 million motile sperm are obtained from the ejaculate. Meanwhile, if <2 million motile sperm are found in the ejaculate, then the Intracytoplasmic Sperm Injection (ICSI) procedure is recommended to be performed.[8]

Studies assessing the relationship between parameters in sperm analysis and the MOST ratio have not been performed to date. This study was conducted to assess whether standard parameters found in sperm analysis such as volume, concentration, motility, and morphology are associated with the MOST ratio in infertile men

MATERIALS AND METHODS

Study Design

This study is a descriptive-analytic study, conducted by taking medical records of infertility patients who underwent sperm examination and sperm function tests as basic routine examinations at infertility clinics, Sadewa IVF, Yogyakarta Province, Indonesia. A total of 864 sperm analysis and sperm survival test data were included in this study from May 2015 to November 2020. The data taken is from patients' medical records who carry out sperm analysis based on WHO manual 2010 and modified sperm stress test.[3, 6] The MOST were carried out at the andrology laboratory, infertility clinic, Sadewa IVF, Yogyakarta Province, Indonesia with the following procedure: Prepare 0.8 mL 90% media [90% media contains of 0.9 mL SpermGrad (SpermGrad^{TM}, Vitrolife, Sweden) and 0.1 mL SpermRinse (SpermRinse^{TM}, Vitrolife, Sweden)] into sterile 5 mL conical tube; Add an amount of 1 mL ejaculate into the conical tube containing 0.8 mL media; Centrifuge in the 1000 rpm in 20 minutes; Remove the supernatant until remaining about 0.2 mL pellet; Transfer the pellet to the new sterile microtube containing 0.5 mL SpermRinse (SpermRinse^{TM}, Vitrolife, Sweden) and homogenized; Take a small amount of sample and count the sperm motility using the Neubauer counting chamber under the inverted microscope with a magnification of 40x; The microtube containing SpermRinse and the pellet was then

incubated at 40° C and counted in every 1, 2, 3, and 4 hours.

Ethics approval and consent to participate

All research procedures are carried out based on the applicable regulation. Secondary data from patient medical records were used in this study. Informed consent was obtained from the patients as participants in this study. Access to medical record data has been approved by the hospital management by maintaining data security. Data security is carried out by providing a code for each patient's data and not displaying the patient's identity. Ethical clearance was obtained from the Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Public Health, and Nursing Universitas Gadjah Mada-DR. Sardjito General Hospital. The protocol number of the clearance is KE/FK/0434/EC/2021 dated May 5, 2021.

Statistical Analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences for Windows, version 26.0 (SPSS, Inc., Chicago, IL, USA). The Spearman rank correlation analysis was carried out to test the correlation between the sperm parameter and the MOST ratio. The results of statistical tests are presented in the form of a correlation coefficient (r).

RESULTS

This study analyzed 864 medical records data of patients with male infertility in an infertility clinic in Yogyakarta Province, Indonesia. As presented in table 1, the mean sperm volume and concentration of the 864 patients were 3.04 mL and 35.74 x 10^6 / mL respectively. The mean forward motility was 47.93%, the mean normal morphology was 2.07%, the mean leukocyte cell number was 0.84%, and the mean MOST ratio was 0.51. Sperm volume was classified as <1.5mL and ≥1.5mL groups. In the <1.5mL group, 20 patients showed a MOST ratio of <0.39 and 38 patients with ≥0.39. Besides that, in the ≥1.5mL group, a number 201 patients had a MOST ratio of <0.39, and 38 patients with ≥0.39 (Table 2).

The sperm concentration was divided into 2 groups, was <15x106/mL and ≥15x106/mL groups. A total of 91 patients in the <15x106 / mL group, showed a MOST ratio of <0.39, and 117 patients with a MOST ratio ≥0.39. The forward motility rate category was divided into <32% and ≥32%. A number of 84 patients with a motility rate <32% had a MOST ratio <0.39, and another 73 patients had a MOST ratio ≥0.39. In the ≥32% forward motility group, there were 137 patients with a MOST ratio <0.39, and 570 patients with a MOST ratio ≥0.39. Based on statistical analysis, there was a significant positive correlation between sperm concentration and forward motility rate with the MOST ratio. The coefficient correlation was 0.235 and 0.302 respectively (Table 2).

Patient data were categorized into 2 groups based on sperm morphology, as well <4% and ≥4% groups. A number of 203 patients with a morphology rate <4% had a MOST ratio <0.39 and the other 568 patients had a MOST ratio ≥0.39. In the ≥4% morphology group, there were 18 patients with a MOST ratio of <0.39, and 75 patients with a MOST ratio of≥0.39.

The number of leukocytes in the ejaculate was categorized into 2 groups, were <1 x 106/mL and ≥1 x 106/mL groups. A total of 177 patients in the <1 x 106/mL group had a MOST ratio <0.39, and 466 others had a MOST ratio ≥0.39. Besides that, in the ≥1 x 106/mL group, a number of 44 patients had a MOST ratio of <0.39, and 177 patients had a MOST ratio of ≥0.39. According to the correlation analysis, the number of leukocytes showed a negative correlation with the MOST ratio and the correlation coefficient was -0.076 (Table 2).

DISCUSSION

This research was conducted using a large amount of data, so it is expected to provide an overview of the relationship between the MOST and sperm parameter analysis. The study results showed a positive correlation between sperm concentration and motility with the MOST ratio. The higher the sperm concentration and motility, the higher the MOST ratio. Meanwhile, the leukocyte parameter showed a negative correlation with the MOST ratio. The higher the number of leukocyte cells in the ejaculate, the lower the MOST ratio. Standard evaluation of sperm analysis in the laboratory to date is based on WHO 2010 manual procedure. The evaluation is carried out by examining the volume, concentration, motility, morphology, number of leukocyte cells, and several other parameters of the patient's ejaculate.[3]

The WHO 2010 manual procedure parameters were taken from semen samples of 4.500 fertile men (men with partners who were pregnant within ≤ 12 months) in 14 countries spread over 4 continents.[9] Sperm analysis is a part of male infertility management that is related to fertility but cannot directly describe the fertilization function of the spermatozoa.[4] This assay cannot describe the potential of spermatozoa to fertilize an ovum or undergo maturation to achieve fertilization.[10] Based on this limitation, the sperm analysis assay needs to be complemented with other laboratory tests to predict sperm fertilizing ability. There was the sperm function test, which was developed as a research tool and not part of routine sperm examination in the laboratory. This test aims to evaluate each different stage of the fertilization process.[11] Although some of the tests on this assay were highly correlated with in-vitro fertilization, sperm function tests are technically and time-consuming, so they are rarely performed as routine laboratory tests.[12]

Calamera et al. has developed laboratory techniques namely modified sperm stress test (MOST) as one of the sperm function tests that aims to predict fertilization in assisted reproductive technology.[6] The difference between MOST and the previous sperm stress test by Alvarez et al. is the shorter incubation time and the intrinsic reaction mechanism. The previous method used a human tubal fluid medium that lacked transitional metal supplementation. The presence of transitional metal supplementation will compromise sperm function. Ham's F10, a culture medium that is already known to contain 52 separate constituents, including transitional metal supplements, was used in the MOST because it might be more related to stimulated lipid peroxidation production compared to the previous method.[5, 6]

A previous study showed that the quality of the media used in sperm preparation in the andrology laboratory will affect the quality of the sperm which in turn will affect the outcome of ART.[13]

The MOST ratio was statistically significantly correlated with the fertilization rate in patients undergoing IVF. The threshold ratio of the MOST ratio that was significant to the fertilization rate was 0.39. A MOST ratio of more than 0.39 is associated with better in vitro fertilization rates.[6] Sperm abnormalities are subjected as the focus of evaluation in the cases of male infertility. Several examinations need to be done to determine spermatozoa's ability to fertilize. There were many aspects evaluated related to sperm parameters, including concentration, motility, morphology, DNA damage, oxidative stress, and various genomic and proteomic compositions.[14] The basic principle of the MOST is to assess sperm motility, which is correlated with various factors that affect the success of ART such as sperm DNA fragmentation and oxidative stress.[7] Sperm ability to move straight forward becomes one of the essential parameters affecting the success of the fertilization. The previous study has demonstrated that a low number of motile spermatozoa had reduced the fertilizing ability and affected the embryo quality during ICSI treatment in severe oligozoospermia.[15]

The presence of leukocytes >1 million/mL of semen is a pathological condition known as leukocytospermia.[3] A previous study by Pratap et al. showed that semen samples with leukocytospermia >1 million/mL had a significant reduction in sperm motility and vitality parameters.[16] Meanwhile, the 0.5-1 million/mL leukocytospermia group showed a significant decrease in motility parameters. DNA damage in both leukocytospermia groups was higher than in the non-leukocytospermia group (normal group). A major component of seminal leukocytes consists of neutrophils and macrophages, which may be the cause of the sperm damage through the generation of ROS and apoptosis induction.[17] Male infertility is often associated with increased DNA fragmentation and poor sperm viability.[18] DNA fragmentation and sperm viability are closely related, with the finding of high numbers of necrospermia in men with high levels of DNA fragmentation.[19] Previous studies have shown a negative correlation between sperm parameters and the DNA Fragmentation Index (DFI). Significantly lower sperm concentration, motility, and normal morphology in the abnormal DFI group (DFI 30%) than in the normal group (DFI < 30%).[20] The presence of oxidative stress due to high levels of leukocytes in sperm has a correlation with decreased sperm motility.[21] This is in line with the results of research conducted with a negative correlation between the MOST ratio and sperm leukocyte levels. The sample group with high levels of leukocytes in the sperm showed a lower MOST ratio.

Previous study conducted by Hatchel et al. revealed the presence of the SLIRP gene which is an RNA-binding protein on steroid receptor RNA activator (SRA).[22] This gene is known to be associated with male infertility as shown in a study on male SLIRP knocked out (KO) mice. In this study, it is known that SLIRP KO male mice have poor sperm motility, and affect their fertilization outcome when crossed with wild-type (WT) females.[23] These several studies prove that sperm motility has a critical role in the fertilization process, and laboratory assays to assess the sperm motility status of infertile patients are important to predict fertilization outcomes in ART procedures.

CONCLUSION

Several studies have declared that sperm motility has an important role in the ART process indicated by the correlation between motility and various factors that affect the outcome of fertilization. As far as we know, this is the first observational study using a big sample size and included all the male infertile patient, both those who will undergo IVF procedures and non-IVF patients. Based on this study, it was known that the MOST ratio has negative correlation with the number of leukocyte cells in sperm but positive correlation with sperm concentration and motility.

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