Predicting the value of sperm analysis using an electronic nose

Aa Koesoema Wijaya[1], Ahmad Kusumaatmaja[2], Dicky Moch Rizal[3]
[1]Departement of Biomedical Engineering, Multidiciplinary Graduate School Faculty, University of Gadjah Mada, Yogyakarta, Indonesia
[2]Department of Physics, Faculty of Math and Natural Sciences, University of Gadjah Mada, Yogyakarta, Indonesia
[3]Department of Physiology, Faculty of Medicine, Public Health, and Nursing, University of Gadjah Mada, Yogyakarta, Indonesia

Article Info

ABSTRACT

Total motile sperm count and DNA fragmentation Index are two parameters in sperm analysis that have recently been used to determine the outcome of the management of cases of male infertility. Total Motile Sperm Count is one of the values considered better than the 2010 World Health Organization standard sperm analysis in terms of predictive value for the success of the spontaneous ongoing pregnancy rate. High DNA Fragmentation Index values were associated with lower pregnancy success and an increased risk of low fertilization rate or total fertilization failure. In this study, we developed a method to classify sperm analysis based on total motility sperm count and DNA Fragmentation Index values by using an electronic nose. In the total motility sperm count (TMSC) study, we use four algorithms with the result of accuracy values 95% and in the DNA fragmentation Index study, we get a fairly good accuracy value for two algorithms with the accuracy values 70%.

Corresponding Author:

Dicky Moch Rizal
Department of Physiology, Faculty of Medicine, Public Health, and Nursing, University of Gadjah Mada
Street of Farmako, North Sekip, Yogyakarta, Indonesia
Email: drdickyandrologi@ugm.ac.id

1. INTRODUCTION

Artificial intelligence has now developed in helping sperm morphology analysis through image processing. The computer-assisted sperm analysis (CASA) can be processed in real-time. However, this CASA examination is quite expensive, and it has not been included in the 2010 WHO guideline for sperm analysis. Another semen examination method that has utilized artificial intelligence technology is calculating sperm concentration using machine learning-based spectroscopy [1]. Semen analysis is the main procedure in infertility examination to classify sperm samples as having normal or abnormal values. Furthermore, the sperm analysis will be used to select, track and collect healthy sperm for in vitro processing. One study applied artificial intelligence to microscopic images of sperm analysis results to simplify and speed up the process of classifying sperm cells using the faster region convolutional neural network (FRCNN) with elliptic scanning algorithm (ESA). This new method can detect sperm and identify sperm motility within 1.12 seconds with an accuracy of 97.37% [2].

Sperm morphology is a very important factor in the assessment of sperm quality. Various artificial intelligence methods have been developed to help classify sperm deformities based on datasets of sperm morphology. One of these studies applies multi-stage cascade-connected preprocessing and machine learning based on a non-linear support vector machine (SVM) kernel. The results of this study provide an accuracy of 86.6% for the human sperm head morphology (HuSHeM) dataset and 85.7% for the Sperm Morphology image data set (SMIDS), respectively [3]. Another study developed an artificial intelligence algorithm using a network-based deep transfer learning approach and deep multi-task transfer learning (DMTL), to classify

sperm heads, vacuoles, and acrosomes as normal or abnormal. This technique can classify all parts of sperm and increase the accuracy of the head, acrosome, and vacuole by 84.00%, 80.66%, and 94.00% on the label [4].

Infertility in men is a health problem that is still common in the community. Various attempts to increase the outcome of infertility management have led to the parameters of the success rate of pregnancy in couples. Total motility sperm count (TMSC) is one of the values considered better than the 2010 WHO standard sperm analysis in terms of predictive value for the success of the spontaneous ongoing pregnancy rate (SOPR). The TMSC value was obtained from the multiplication of the volume of seminal fluid, sperm concentration, and the percentage of the motility values of spermatozoa. In addition, TMSC also plays a role in increasing the predictive value of pregnancy outcomes in couples undergoing intracytoplasmic sperm injection. A study showed the normal total motile sperm count group had a higher fertilization rate (p=0.016) and lower miscarriage rate (p=0.041) compared to the abnormal total motile sperm count group [5], [6].

A study that used the parameters of processed TMSC and 24-hour sperm survival as predictors, showed that the processed TMSC value $>10 \times 10^6$ and the 24-hour sperm survival value $>70\%$ were good predictors for the success of IUI, while the processed TMSC value $<5 \times 10^6$ and the 24-hour sperm survival $<30\%$ is a poor predictor. However, the pattern of TMSC values from time to time is also quite important to know considering that increasing age and giving therapy in cases of infertility will also affect the trend of TMSC values that occur. In a meta-analysis examining the age relationship, TMSC scores and grouping of TMSC groups by year showed that the proportion of men with normozoospermic decreased, and the risk of needing infertility treatment in men increased over the study period. Although the study method used is retrospective, the shift in the group of men requiring clinical infertility treatment is quite relevant. Thus, it can be concluded that the TMSC value has an important role in the prognosis of infertility therapy. In contrast to the above study, another study showed that TMSC had no significant effect on IUI success in patients with unexplained infertility. In that study, the outcome parameters were live birth rate (LBR) and clinical pregnancy rate (CPR) [7]–[9].

DNA fragmentation index (DFI) is one of the most important parameters in assessing male infertility. DFI correlated with the level of infertility and levels of microelements in the serum such as zinc and magnesium. It is also known that high levels of reactive oxygen species (ROS) in semen plasma contribute to the formation of sperm DNA fragmentation. The study showed that DNA damage had an impact on the low motility of sperm cells. However, DFI is not related to conventional sperm analysis parameters. Another study using correlation analysis showed that DFI was positively correlated with age, abstinence period, semen volume, and abnormal morphology of spermatozoa cell heads meaning that the higher the number of these parameters, the higher the DFI value. On the other hand, DFI was negatively correlated with sperm concentration, sperm progressive motility, and percentage of normal sperm morphology. This is due to the results of studies showing an increase in sperm concentration, sperm motility, and sperm morphology after varicocelectomy, and has been shown to reduce DFI [10]–[12]. The role of the DFI examination as a means of predicting the outcome of IUI has recently grown worldwide, as evidenced by a meta-analysis and systematic review that a low DFI value in pregnancy after IUI has a relative risk value of 3.15, with a sensitivity of 94% and specificity 19%. However, further research is still needed to assess the relevant and precise DFI cut-off value and the stability of the test results obtained from time to time [13].

The idea used in this study is to utilize metabolomic gases that form pattern recognition of volatile organic compounds produced from the smell of sperm fluid. The metabolomics study of semen can be used for the development of electronic nose technology for diagnostic support in infertility cases. The use of gas chromatography/mass spectrometry (GC-MS) technology for routine clinical use is considered quite expensive and time-consuming, so the use of electronic nose technology can be used as a cheaper and faster alternative. The electronic nose will interpret the smell of sperm fluid through a series of certain gas sensors to be further converted into electrical signals and analyzed with the help of artificial intelligence algorithms. The selection of feature extraction methods in electronic nose technology plays an important role in increasing accuracy. Several feature extraction methods are currently widely used for electronic nose development including window time slicing (WTS) moving window time slicing (MWTS), moving window function capture (MWFC), curve fitting parameters, transform domains, phase space (PS), and dynamic moments (DM), parallel factor analysis (PARAFAC), energy vector (EV), and power density spectrum (PSD). This study aims to develop electronic nose technology in detecting the characteristics of semen and classifying it based on the clinical cut-off value of DFI [14]–[16].

2.   METHOD

This study used semen samples from 98 male patients with suspected infertility at the fertility clinic of Sadewa Hospital, Yogyakarta, Indonesia. The sample is taken simultaneously from patients who will perform sperm analysis with the masturbation process at the fertility clinic after abstinence for 2-7 days. In addition, this study also took semen samples from patients with an indication of DNA fragmentation index (DFI) examination of 21 subjects. The sperm analysis method used is the conventional technique. The sperm

analysis was carried out at the laboratory of Sadewa Hospital, Yogyakarta. The remaining seminal fluid samples were stored in a refrigerator with closed containers. The process of measurement is carried out using the electronic nose (GeNose) tool in the Materials and Instrumentation Physics Laboratory of the Physics Department, Faculty of Mathematics and Natural Sciences, Gadjah Mada University.

As shown in Figure 1, the examination was carried out by placing 0.3 cc of the sample using a pipette in a 100 cc beaker with a tight lid, then a hose connected to the e-nose was installed. The sampling process is carried out at room temperature, with a total period of the test is 60 seconds. The gas sensor array pattern is recorded using data logger software. We use several types of values for feature extraction which are then followed by a supervised classification algorithm. The greater the value of TMSC means the better the motility quality and the lower the value of DFI means the less damage to the DNA of spermatozoa cells.

Figure 1. Method of examining semen samples using an electronic nose and beaker tube with a modified lid

We divided the TMSC study subjects into three groups based on the TMSC value, namely the 'less than 30' group, 'between 30-60' and 'more than 60' groups. The division of the TMSC category was based on the reference of previous research which divides the TMSC value into 2 parts, namely 'less than 20' and 'more than 20' to assess the success of 'spontaneous pregnancy rate' after intra uterine insemination (IUI) treatment [4]. We also divided the DFI study subjects into two groups based on the DFI value used as a cut-off, namely the 'equal or less than 15' group, 'between 16-25' group, and the 'equal or more than 25' group. Based on a reference from previous studies which divided the DFI value into 'less than 30' and 'more than 30' as parameters to assess the outcome of intracytoplasmic sperm injection (ICSI) treatment [17].

We use five kinds of feature extraction values at 10th to 60th seconds of data, namely: gradient, max, min, mean, median, std, trapz and var. Data preprocessing with minmax scaler on the dataset for data normalization. The stratified 3 fold and 5 fold cross validation method is used during the machine learning process with the distribution of 30% testing dataset and 70% training dataset. The machine learning algorithms used are random forest (RF), k-nearest neighbors (kNN), support vector classification (C=1, gamma= 'scale', kernel= 'rbf') and linear discriminat analysis (LDA). For data analysis, we use Python based software.

3. RESULT AND DISCUSSION

This research is a preliminary study to develop electronic nose technology in classifying several parameters resulting from sperm analysis such as total motile sperm count (TMSC) and DNA Fragmentation Index (DFI). These two parameters are often used in clinical practice to help predict the success of assisted reproductive technology. The characteristics of the TMSC research subjects in Table 1 show that the average age of the subjects of the preliminary study was 32.47 years. The length of storage of samples in the laboratory refrigerator is also quite varied with an average of 5.58 days. The abstinence period of the study subjects was also in accordance with the protocol for examining sperm analysis with an average abstinence period of 3.94 days. The total volume of sperm fluid produced by the research subjects was quite varied with the average volume of semen being 3.16 cc. As it is known that research on volatile organic compounds in semen has been carried out several times using gas chromatography and mass spectrometry methods, however, the pathophysiological process that underlies a disease related to infertility is important to classify the results of these metabolomics studies [18]–[20]. The characteristics of the TMSC research subjects are as follows.

Table 1. Characteristics of TMSC research subjects (98 subjects)

No	Parameter	Category	Amount	Average Value
1	Ages	< 30 years	29	
		30 - 34 years	35	
		35 - 39 years	26	
		40 - 44 years	7	32.47 years
		45 - 49 years	0	
		50 - 54 years	1	
2	Storage Time	< 3 days	20	
		3 - 6 days	40	5.58 hari
		7 - 14 days	38	
3	Abstinence Period	< 4 days	40	
		4 - 5 days	45	3.94 hr
		6 - 7 days	13	
4	Cement fluid volume	< 1 cc	2	
		1 s.d 2 cc	46	
		3 s.d 4 cc	42	3.16 cc
		5 s.d 6 cc	7	
		≥ 7 cc	1	
6	Total Motility Sperm Count	>60	36 (36.7%)	
		30-60	18 (18.3%)	50.95
		<30	44 (44.8%)	

The process of classifying semen for TMSC and DFI values is usually carried out by direct observation of several sperm analysis parameters under a microscope, which requires more time and accuracy. The development of electronic nose technology aims to further accelerate the classification process by recognizing the pattern of metabolomic gases detected from semen. Thus, it is hoped that it will facilitate and speed up the fertilization process using assisted reproductive technology, especially in the selection of spermatozoa cells to be used. Several important parameters play a very important role in assisted reproductive technology, including pregnancy and birth rates after in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), sperm genetic integrity, and fertilization capacity [21]–[23]. The characteristics of the DFI research subjects are shown in Table 2 and Table 3.

Table 2. Characteristics of DFI research subjects based on sperm analysis (21 subjects)

No	Parameter	Average value
1	Ages	34.14 years
2	Volume	3.2 cc
3	Viability	71.57%
4	Concentration	26.54 million cells/cc

Table 3. Category of DFI group based on sperm analysis (21 subjects)

No	DFI category	Amount
1	equal or less than 15	9 (42.8%)
2	between 16-25	6 (28.5%)
3	equal or more than 25	6 (28.5%)

The feature extraction of the mean, median, max, min, std, trapz and var values can be done using the dimensional reduction method using PCA with a total value of explained variance above 75%, as shown in Figure 2. In the TMSC study, we get a fairly good accuracy value for several algorithms (RF, kNN, SVM, LDA) both on training accuracy and on testing accuracy. The best results in this study showed an accuracy value of 0.95, precision 0.97 and recall 0.92 for the random forest algorithm, as shown in Figure 3. This result supports the theory which states that sperm quality is directly proportional to an increase in TMSC value and a low DFI value because a high TMSC value will increase the probability of fertilization by sperm with good motility and a low DFI value correlates with a decreased risk of recurrent spontaneous abortion [24]. TMSC values also correlated with live birth rates in infertile couples. Another randomized, prospective, and multicenter study investigated the association between the performance characteristics of the intrauterine insemination (IUI), post-processing TMSC method and the live birth rate in couples with unexplained infertility. The results of this study indicate that patient discomfort with the IUI method and low TMSC scores are associated with lower live birth rates. The preparation process for IUI and guidance with ultrasound during IUI were also not related to the success rate of live births. Thus, it can be concluded that the high TMSC value of the success rate of live births in unexplained infertile couples. A similar study examined the predictive value of TMSC for spontaneous pregnancy and pregnancy after IUI, suggesting that TMSC values >5x10^6 per ejaculation require treatment with IUI. This is also supported by the results that the TMSC value of 1-5x10^6 resulted in a lower spontaneous pregnancy rate compared to those who received IUI treatment [25].
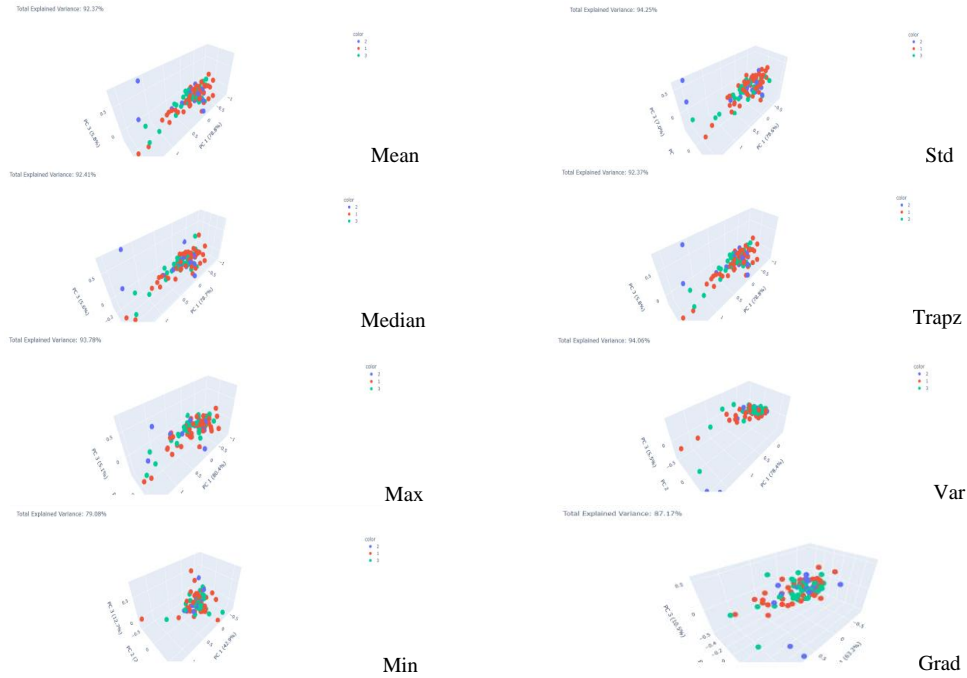
Figure 2. The visualization of 3D PCA (mean, median, max, min, std, trapz, var and grad)
features extraction on TMSC dataset with a total value of explained variance above 75%
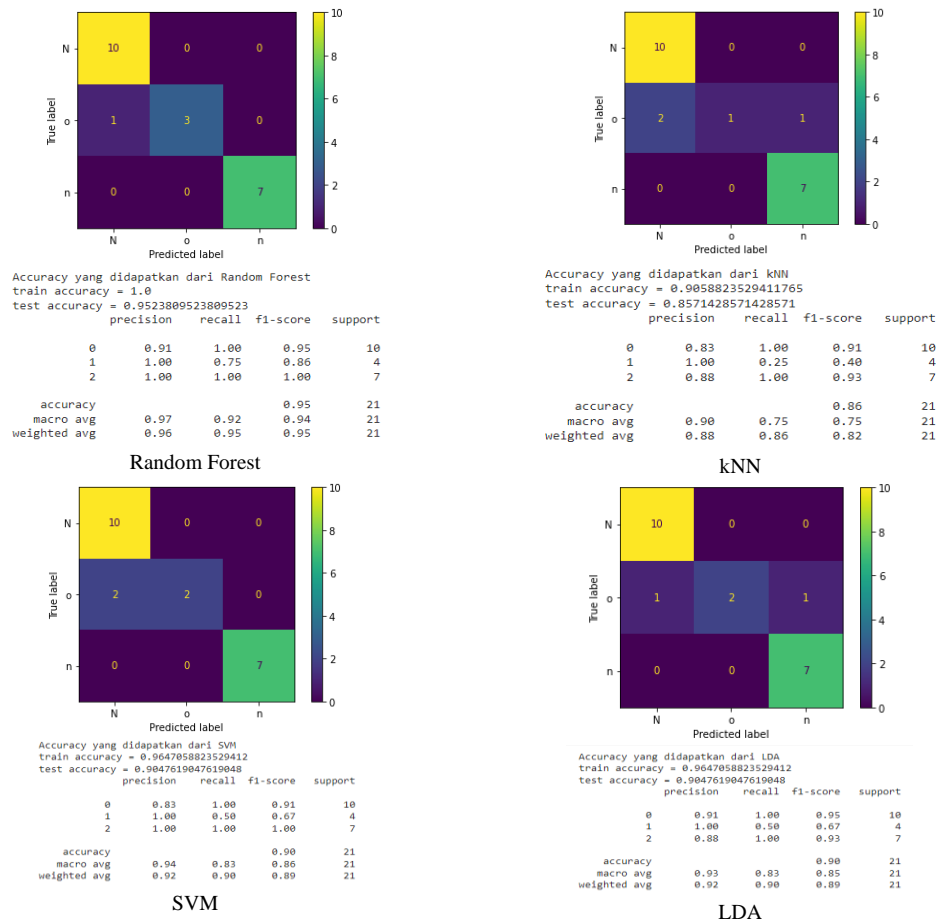

Figure 3. Comparison of the confusion matrix in the TMSC classification dataset using stratified
5-fold cross-validation on several algorithms (RF, kNN, SVM and LDA)

The feature extraction of the mean, median, max, min, std, trapz and var values can be done using the dimensional reduction method using PCA with a total value of explained variance above 80%, as seen in Figure 4. In the DFI classification study, we get a fairly good accuracy value for several algorithms (RF and LDA) both on training accuracy and testing accuracy. The best results in this study showed an accuracy value of 0.71, precision 0.67 and recall 0.75 for the random forest algorithm, as shown in Figure 5. The DFI examination is important in predicting low outcomes or failure of in vitro fertilization (IVF). A retrospective study proved that for cases of infertility in men with mild to moderate asthenozoospermia, high DFI values were associated with lower pregnancy success and an increased risk of low fertilization rate (LFR) or total fertilization failure (TFF). Thus, the DFI value can be used as an outcome predictor in IVF action. Another benefit of the DFI examination is to identify risk factors for recurrent abortions that cannot be explained. A study describes the relationship between DFI values and the incidence of recurrent spontaneous abortion, where a high DFI value increases the cases of unexplained recurrent spontaneous abortion. In addition, other studies have shown that high DFI values are positively correlated with IL-8 levels and negatively correlated with spermatozoa cell vitality in cases of infertility patients with leukospermia. This illustrates that there is a relationship between leukospermia and the incidence of DNA damage in sperm cells, which is thought to be due to high levels of malondialdehyde (MDA) due to the oxidative stress process [26], [27].

However, DFI examination requires a high cost and takes a long time, so further research is needed that can provide the same good outcome as DFI examination with an efficient method. One of these efforts is to utilize the metabolomic study of volatile organic compounds produced by sperm fluid. A study of metabolomic markers of semen from patients with infertility due to oligozoospermia demonstrated the potential of several biomarkers that were positively correlated with the motility of spermatozoa cells. The study used nuclear magnetic resonance spectroscopy with multivariate statistical analysis. In addition, studies of the prostatosome related to infertility have also been carried out, where the prostatosome is an extracellular vesicle secreted by prostate gland cells during ejaculation which consists of various proteins and plays an important role in the field of fertility-related immunology [10], [19].
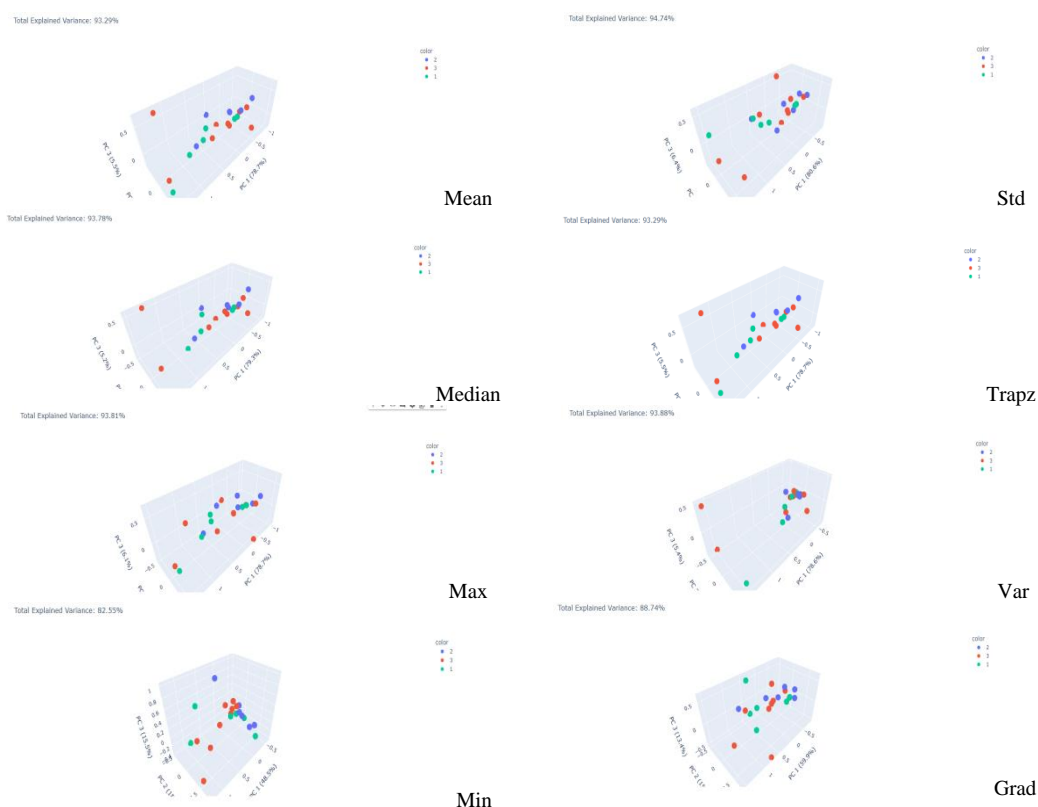

Figure 4. The visualization of 3D PCA (mean, median, max, min, std, trapz, var and grad) features extraction on DFI dataset with a total value of explained variance above 85%

This study has several weaknesses, including the absence of validation of the TMSC and DFI cut-off values set by WHO to serve as clinical guidelines, so that the grouping in this study is only based on the estimated value of previous studies [9]. In addition, the number of samples used in this study is still very

limited due to the limited time of the study and the high cost of the examination, so that more data is needed to improve the accuracy of the research results. The duration of storage of semen samples in this study varied in the refrigerator, so it did not rule out the possibility of changes in metabolomic gases due to the growth of contaminant bacteria. In addition, it is necessary to experiment with other feature extraction models to increase the accuracy obtained [28]-[30].
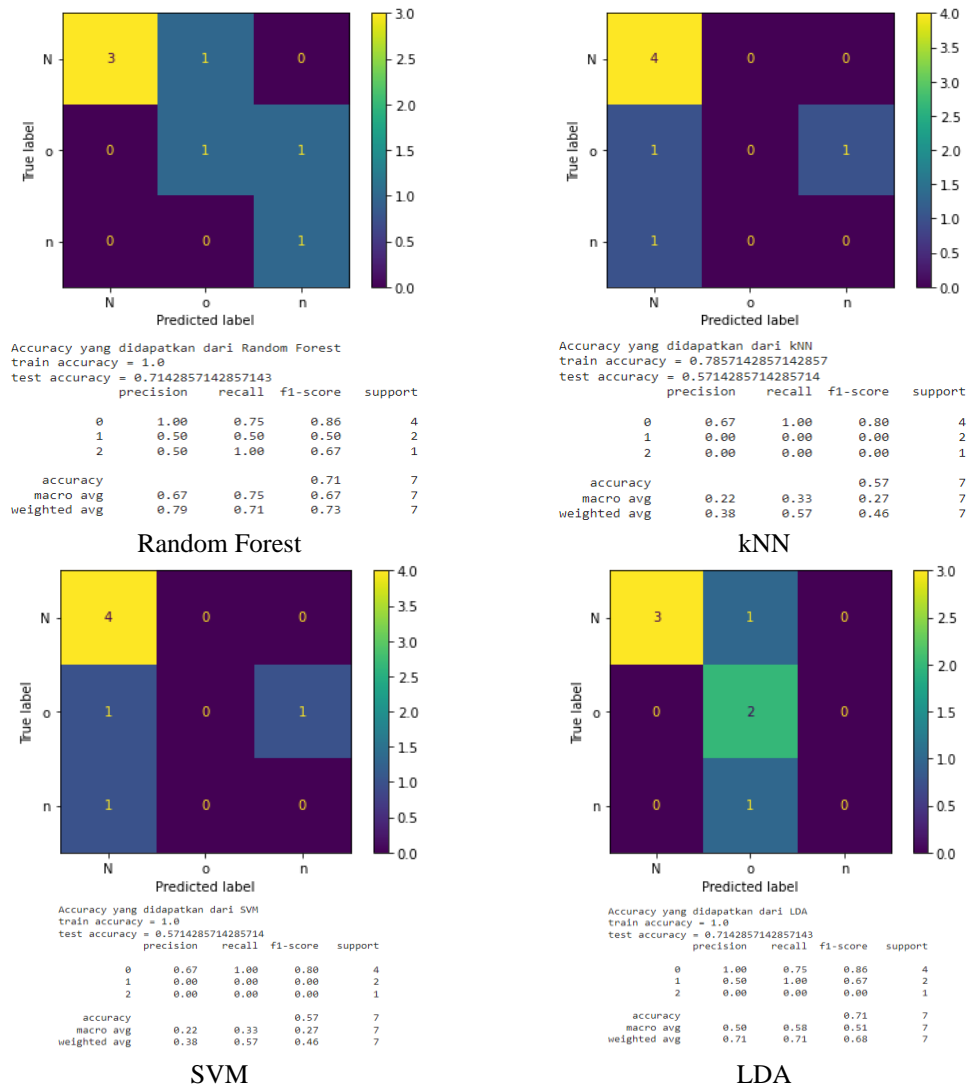
Figure 5. Comparison of the confusion matrix in the DFI classification dataset using stratifed 3-fold cross-validation on several algorithms (RF, k-NN, SVM and LDA)

4.   CONCLUSION

It can be concluded that the use of electronic nose technology to classify and predict the result of sperm analysis has good potential to be developed. The TMSC and DFI values, which are currently widely used in clinics to predict the outcome of infertility therapy with assisted reproductive technology, still need to be studied further using gas chromatography and mass spectrometry, especially in terms of metabolomic studies among several groups of values being compared. This is necessary to facilitate the application of electronic nose technology as a rapid diagnostic tool. In addition, further experimentation is still needed on the extraction model that can provide the best accuracy from the results of the GeNose dataset for varicocele cases. One of the suggestions is to analyze the test results of samples from fertile men and then compare them with test results on men with varicocele infertility and non-varicocele infertility.

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BIOOGRAPHIES OF AUTHORS

dr. Raden Aa Koesoema Wijaya, M.Sc. ⓘ 🇬 sc Ⓟ was born in Cilacap, July 25, 1986. He currently works as a civil servant at the North Seribu Islands Health Center and is pursuing a master's degree in biomedical engineering at a graduate school, Gadjah Mada University. The research areas of interest are biomedical instrumentation, biomaterials, and artificial intelligence. He can be contacted at email aa.koesoema.wijaya@mail.ugm.ac.id.

Dr.Eng. Ahmad Kusumaatmaja, M.Sc. ⓘ 🇬 sc Ⓟ was born in Klaten, July 22, 1983. Currently, Dr. Ahmad Kusumaatmaja, M.Sc. has an office at the Department of Physics, Faculty of Mathematics and Natural Sciences, Gadjah Mada University, Sekip Utara PO BOX BLS.21 Yogyakarta, 55281. Dr. Ahmad Kusumaatmaja, M.Sc. completed his bachelor's and master's degrees from Gadjah Mada University, in the field of Physics and continued his doctoral education at the Nara Institute of Science and Technology, Japan by taking the Materials Science field. Several studies related to biomaterials have been carried out including "Solvent vapor treatment improves mechanical strength of electrospun polyvinyl alcohol nanofibers" (2018), "Polyacrylonitrile nanofiber-based quartz crystal microbalance for sensitive detection of safrole" and "Chitosan-based quartz crystal microbalance for alcohol sensing" (2018). The most recent studies is optimization of the "Machine Learning" System For Early Detection Of Covid-19 Through Electronic Nose [2020]. He can be contacted via email ahmad_k@ugm.ac.id.

Dr. dr. Dicky Moch Rizal, M.Kes, SpAnd(K), AIFM., ⓘ 🇬 sc Ⓟ born in Surabaya, October 8, 1969, is a physiology lecturer who has taught at the Faculty of Medicine, Public Health, and Nursing (FKKMK) Universitas Gadjah Mada (UGM) since 1996. The author is the Head of the Department of Physiology, FKKMK UGM since 2017 until now. Activities other than teaching are staff at the Permata Hati Infertility Clinic, RSUP Dr. Sardjito, Yogyakarta; Head of Lab Gladiool IVF, RSIA Gladiool, Magelang; and Head of the Andrology Lab at Sadewa IVF RSKIA Sadewa. He received his medical education at Diponegoro University (Undip) Medical Faculty Semarang (1988-1994), Masters in Reproductive Biomedical at the Postgraduate Program at Undip Medical Faculty Semarang (1998-2003), National Board Andrology Specialist at Unair Medical Faculty (2006), Masters education 3 in the Postgraduate Program of Faculty of Medicine UGM (2009-2015), and Consultant Andrology Specialist for Fertility and Reproductive Endocrinology (2019). As an Andrology Specialist who also handles IVF, the author also actively participates in several trainings, such as IVF Training at the National University Hospital Singapore (2011), IVF Training at Van Hahn Hospital Ho Chi Minh Vietnam (2013), and Total Quality Management of IVF Training. Laboratory in Bangkok (2019). His research fields include endocrinology and human reproductive systems, andrology, cell culture, aging, and assisted reproductive technology. During his time as a lecturer, the author has published several scientific works. Recent publications by the authors include "Effect of Gamma-Mangostin on Testosterone Levels in Leydig Cell Culture of Sprague-Dawley Rat Induced by Advanced Glycation End Products: A Preliminary Study" (2019); "Stimulatory Effect of Methanolic Extract and N-hexane Insoluble and Soluble Fraction of Parijoto Fruit (Medinilla speciosa Blume) on The Spermatozoa Quantity of Male Sprague-Dawley Rats" (2020); and "The Effect of Black Pepper Fruits (Piper nigrum L.) on The Increase of Erection" (2020). He can be contacted via email drdickyandrologi@ugm.ac.id.