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Differences of Spermatozoa Concentration Analysis Between Manual and Automatic Methods

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Abstract

The examination of sperm concentration in the laboratory is the calculation of the number and motility using a microscope or using a device. There are still some clinicians who doubt the accuracy of the sperm count results using a semen analyzer rather than using the manual method. This study aims is to determine the differences of the sperm concentration examination between the manual method and the automatic method. Subjects in this study were patients who carried out semen analysis tests at the Clinical Pathology Laboratory of RSIA "Restu Ibu" Sragen from June to August 2020. The object of this research is the examination of sperm concentration, using a manual method using a hemocytometer and an automatic method using the LensHooke ™ SQA X1 Pro. The results of statistical tests using the Mann Whitney methods show that the significance value (p) was 0.960, which means that there was no difference in the results of the sperm concentration examination between the manual method and the automatic method. Result of this research shows that there is no weakness or significant difference if compared between manual and automatic methods.

Keywords

Spermatozoa, Semen Analyzer, Manual Spermatozoa, LensHooke SQA X1 Pro, Hemocytometer.

INTRODUCTION

The infertility rate for married couples worldwide according to the World Health Organization (WHO), it is estimated that 10-15% of couples who experience infertility problems during the reproductive period. Male factors alone account for about 20% of infertility cases and a combination of male and female factors accounts for about 50-60% of all infertility cases (1). Semen analysis is one of the initial tests performed on infertility cases to evaluate the male side. The purpose of semen analysis is to determine the condition of sperm, the results can determine whether the man is fertile or infertile (2). Routine evaluation of male infertility cases currently uses semen analysis as a standard covering concentration, motility and morphology (3). The concentration of spermatozoa in the future decreases, therefore examination of the concentration of spermatozoa is very important to establish cases of infertility. Manual method of spermatozoa concentration examination using this simple tool is an examination of spermatozoa concentration that has been recommended by the WHO as the gold standard (2). The semen analysis examination includes macroscopic examination and microscopic examination. The macroscopic parameters of the semen that were examined included volume, appearance, color, coagulation, liquefaction and viscosity. Meanwhile, the microscopic parameters examined from the semen sample include the concentration, motility and morphology of spermatozoa and other cell components (4). In calculating the concentration of the manual method using a hemacytometer counting chamber, it has the advantages of being easy to use, low cost of equipment and operational investment. While the drawbacks require high accuracy, the time required is relatively long between 30 and 60 minutes, is subjective, there are intra and inter-laboratory differences, not all laboratories have other trained verification personnel (5). Along with technological developments in the laboratory field, there is now an examination method for automatic semen analysis. which combines computer technology and has been developing for approximately 40 years. Through the advancement of devices for capturing images from the microscope, a large increase in computing power along with a reduction in computer size, and updated software algorithms are urgently needed (3). In an automatic method based on the development of digital imaging technology to obtain fast sperm concentration results, the reading process only takes 3-5 minutes. The level of accuracy, which has a good correlation and agreement of results overcomes the subjectivity problems of the assessment and is able to improve and standardize the concentration parameters. In its use, the semen quality analyzer tool is still found to

be deficient, including the ability of the tool to not be able to read sperm concentration if the number of spermatozoa is less than 16 per field of view, the investment costs for the equipment and operation of the equipment are quite high (6,7).

There are still some clinicians who doubt that semen analysis uses an automatic method, this further strengthens researchers to carry out and test the quality of this automatic tool with spermatozoa concentration parameters (6,8). Examination of spermatozoa using the automatic method of semen quality analyzer can improve accuracy, one of which is about the results. reading of spermatozoa concentration (9). In the research conducted by Dearing *et al*. (10) the criteria examined were comparisons with the Improved Neubauer and Leja 20-µm spaces, within and between field accuracy, linearity of sperm concentrations from diluted stocks in semen and media, accuracy against internal and external quality materials, assessment of uneven flow effects and receiver. Analysis of surgical characteristics for predicting fertility was compared with the Neubauer method. This work demonstrates that CASA's SCA technology is not a standalone 'black box', but rather a tool for trained staff that enables rapid and multiple sperm counts, providing identified and corrected errors. This system will produce accurate, linear, and precise results, with less analytic variance than the manual method which correlates well to the Improved Neubauer space. In the one of study comparing manual and automatic using the SQA-ICP type, the sample used in the study was 50 samples. The difference in research on the use of the Leenshoke X1 PRO automatic tool and the number of samples analyzed was 40 samples (9). The differences in the above research have sparked the interest of researchers to test different spermatozoa concentrations, using two different methods, namely, the manual method with a computation chamber and an automatic method with a Semen Quality Analyzer (11). The purpose of this study was to compared the examination of sperm concentration, between manual methods using a hemocytometer and an automatic method using the LensHooke ™ SQA X1 Pro. Result of the comparison calculation of sperm concentration between the manual method and the automatic method showed with the Mann Whitney method, the Sig value is 0.960. Because the value of Sig> 0.05, it can be concluded that H0 is accepted and H1 is rejected.

MATERIALS AND METHODS

Spermatozoa analysis includes macroscopic examination by direct observation, which consists of volume, liquefaction (liquefaction), appearance, odor, consistency, viscosity, and pH. While the microscopic examination using a

microscope, which consists of assessment of concentration and motility, agglutination, morphology and vitality of spermatozoa (12,13). Microscopic sperm analysis refers to three parameters, namely sperm concentration or number, sperm motility or movement speed, and sperm shape or morphology. The method of calculating the concentration of spermatozoa starts from the preparation of the patient where special instructions are needed to the patient before releasing the sperm, then the method of collecting sperm which includes a special room for sperm production, containers, labeling, storage, delivery, and examination forms before proceeds with processing and examination specimen (2,14). Hemacytometer chamber to count the concentration of spermatozoa are has 25 large boxes, each large box is divided into 16 small boxes consisting of an upper and a lower side each side.

Semen analysis macroscopic observations without using a microscope but with a visual, examination includes : Liquefaction at room temperature with normal semen thaw within 60 minutes, normal semen appereance is gray white or pearl white, characteristic odor or smell like a flamboyant flower. Consistency and viscosity are liquefied, will drip small and slowly, if they are in the form of tendrils more than 2 cm or do not want to break are hyperviscosity, and if it looks like water and clear it is hypoviscosity. Normal pH value of spermatozoa are 7.2 – 7.8 and the normal volume is 1.5 – 5 mL (15,16).

Microscopic semen analysis filter test is the examination of semen fluid using a microscope. Microscopic observations include assess the concentration and motility of spermatozoa, do homogeneity manually, drop 10 microliters on a slide, cover with a cover glass, allow 1 minute to stabilize, check at room temperature and magnification 400x. Observe the entire rectangular area of the cover slip directed left-right-up-down, if the distribution of spermatozoa is uneven, a new preparation is made. Agglutination, performed when assessing the movement of spermatozoa, observe 10 fields of view randomly, the assessment is: head-head, head-neck, head-tail, tail-tail or mixed, average agglutination is estimated at 5%. Vitality of spermatozoa, the assessment is carried out if the number of moving sperm is more than 30% and to determine the sperm that are actually alive at the time of expulsion (15). Counting the concentration of macroscopic spermatozoa. This research uses observational analytic laboratory, where to find the differences sperm concentration using two methods: the manual method and automated method hemocytometer semen quality analyzer.

The subjects in this study were patients who carried out semen analysis tests at the Clinical Pathology Laboratory of RSIA

"Restu Ibu" Sragen from June to August 2020 with legal letter number 079-17/B/RSIA RI/III/2020. Determination of the sample size of the population used the formula Isaac and Michael (14) according to Sugiono (16).

$$S = \frac{\lambda^2.N.P.Q}{d^2.(N-1)+\lambda^2.P.Q}$$

$$S = \frac{3.481 \times 40 \times 0{,}5 \times 0{,}5}{0.05^2(40-1)+3{,}841 \times 0{,}5 \times 0{,}5}$$

$$S = 35.9700$$

$$S = 36$$

The number of samples obtained by the researchers was 36 samples. Sampling was carried out on respondents who came to be tested for semen analysis who had been educated and promised to come for the examination in sequence.

Inclusion criteria in this research were patients aged 20 to 45 years or reproductive age and patients with spermatozoa yields of more than 20 spermatozoa cells per field of view. Exclusion criteria were hemospermic semen samples or blood-stained semen samples, semen sample with leukospermia, semen sample with many immature sperm cells and semen sample less than 1.5 mL. The independent variables are the manual method of the hemacytometer and the automatic method of the semen quality analyzer. Dependent variable is the result of examination of spermatozoa concentration.

The object of this research is the examination of sperm concentration, using a manual method using a hemocytometer and an automatic method using the LensHooke ™ SQA X1 Pro. The results obtained was calculated statistically with SPSS 20 to determine the difference in the results of the examination between the two methods. This research get approval from The Ethics Commision for Health Research of Muhammadiyah Semarang University.

Manual Method

The reading of spermatozoa concentration, manual method using a counting chamber (Improved Neubauer) consists of an upper and a lower side, each side has 25 large boxes, each large box is divided into 16 small boxes. The calculation is a large box, starting with the middle box (2,17,18). The steps for calculating the concentration by dilution are as follows: A diluent solution is prepared: dissolve 50 grams of sodium bicarbonate ($NaHCO_3$) and 10 mL of 35% formalin in 1,000 mL of aquabidest. Stir the sample until homogeneous. Determine the dilution to be used in the sample by observing the wet preparation: drop 10 µL of homogeneous semen on the object glass, cover with a cover glass (size 22 mm x 22 mm), observe with a 400x magnification light microscope. After determining the dilution to be used, make a tube containing 50 µL of the homogeneous

sample plus a diluent solution. A computation chamber with a cover glass (thickness size 4; 0.44 mm) was prepared. As much as 10 µL sample mixture were taken and a homogeneous diluent solution, then fill it in one of the counting rooms for the improved Neubauer haemocytometer. Let stand for 4 minutes, then count the number of spermatozoa in 10-15 minutes to avoid evaporation. The spermatozoa concentration is counted, until at least 200 spermatozoa have been observed and the rows of the five large squares are fully examined. Record the number of rows when counting reaches 200 spermatozoa. Calculations were carried out in other counting rooms as many as the rows obtained during the first calculation. If the difference between the number of spermatozoa in the two counting chambers is high, the calculation is repeated. If the difference does not differ greatly, the spermatozoa concentration is calculated (19-21).

In the examination of sperm concentration using the manual method, it begins with the preparation of a sample that meets the criteria. The sample is left to stand for a maximum of 60 minutes until it melts (liquefaction), then dilution is carried out and analyzed by dropping the semen liquid into the Improved Neubower counting chamber, the calculation is carried out in 1 large box in the middle. In this study, following standard criteria, the counted cell is the cell in the middle and touches the left and top lines (Figure 1). The cells that touch the right and bottom lines are completely uncountable. The drawback in this manual method is that the sperm cells that are constantly moving make it difficult for visual capture, so it is possible that the cells that should be counted are not counted and vice versa. Table 1 shows table of correction factors calculation of spermatozoa concentration with "improved neubauer" counting chamber.

Figure 1. Spermatozoa in the middle of chamber field (red box)

Determine the required dilution of the initial semen sample and make a wet preparation, to estimate the number of spermatozoa per large field of view (x 200 or x 400) (2).

Table 1. Table of correction factors calculation of spermatozoa concentration with "improved neubauer" counting chamber

Spermatozoa per x400 field	Spermatozoa per x200 field	Dilution required	Semen (µL)	Fixative (µL)	Chamber	Area to be assessed
>101	>404	120(1+19)	50	950	Improved Neubauer	Grids 5,4,6
16-100	64-400	1:5(1+4)	50	200	Improved Neubauer	Grids 5,4,6
2-15	8-60	1:2(1+1)	50	50	Improved Neubauer	Grids 5,4,6
<2	<8	1:2(1+1)	50	50	Improved Neubauer or large-volume	All 9 grids or Entire slide

Source : Laboratory manual for the examination and processing of human semen 5 ed WHO Library (2010)

Automatic Method

This automatic semen analysis was performed using the LensHookeTM X1 PRO (Bonraybio Co., CN) semen quality analyzer for sperm concentration, World Health Organization (WHO). LensHookeTM X1 PRO technology is based on a high-resolution optical lens and built-in autofocus combined with an artificial intelligence autoculation system, the rationale for using autofocus optical lenses to replace laboratory microscopes is based on the concept of using an automated optical inspection system (5). Specifications of the LensHooke™ SQA X1 Pro semen quality analyzer, measuring 140mm wide, 70mm thick, 71.2mm high, 3.5" resistive touch panel control panel, USB 2.0 input and HDMI output, resolution 320 (H) x 480 (V) Color dot matrix, AC100-240V/50-60Hz power supply; DC output 5V/2A rechargeable Li-polymer battery (6). The accuracy of the SCA Semen Quality Analyzer is generally very good with optimal accuracy at 200-600 spermatozoa per field, this is directly from the CASA (Computer-assisted sperm analysis) screen SCA developed for single and multiple plane precision tests. The Semen Quality Analyzer is accurate with its concentration of latex beads. In the semen quality analyzer, quality control is carried out using special reagents, if ten thousand samples of semen analysis are carried out on the automatic semen quality analyzer, or it can be done every six months (12).

The Semen Quality Analyzer provides increased precision over manual methods and can now be applied to routine spermatozoa analysis provided adequate quality control procedures and high measurement standards are followed. Spermatozoa concentration and motility data on semen quality analyzers have been shown to be associated with various fertility measures, although over-calculation of specific semen quality analyzer methods because spermatozoa measurements have been well documented, several systems have been shown to accurately calculate

spermatozoa concentrations (13). The sperm collection device for the Semen Quality Analyzer uses a special cup test consumable. Wait 30 to 60 minutes for sperm to thaw and then homogenized the sample in the cup by turning it back and forth 8-10 times.

Sperm collection device for the Semen Quality Analyzer uses a special consumable test cup. Wait for 30 to 60 minutes for sperm thawing, then the sample is homogenized in the cup by inverting it 8-10 times. Check the color and volume of the sperm sample (Figure 2).

Figure 2. Procedure for a semen cassette quality analyzer.

The first to know is to not touching the double drip test zone inside and outside (under the area), touching the test zone with hands or gloves could contaminate the detection window and will lead to inaccurate results. The correct semen test cassette will be displayed on the monitor, if the cassette is not properly inserted there will be sound. The results will appear on the screen (13).

The technology used artificial intelligence or light to capture in the tool system, apply the sample to the cassette, use the thumb to open the cassette. The concave design of the cassette opening allows analyzing the concentration, motility and morphology of the semen and detecting, analyzing the pH of the semen. Operational way with automatic tool cassette are checked for unexpired test cassette that has not changed color (no green). Do not touching the double-drip test zone inside and outside (underneath the area) Touching the test zone with your hands or gloves can contaminate the detection window, and will lead to inaccurate results. Applying the first pH drop then drop it to the "concentration". Avoid bubbles, don't drip the sample on the top cover of the cassette, then pressing the yellow area to close the cassette. The correct semen test cassette will be displayed on the monitor.

All patients who have come are asked to fill out an informed consent to perform a semen analysis examination and will be educated about preparation, from abstinence to how to remove the sample and collect it.

The way to store spermatozoa so that the results are better is by masturbating without using tools, such as gel, detergent and other aids, may be accompanied by his wife in a special room provided by the hospital, because it will affect the spermatozoa samples that are accommodated and will be analyzed semen. Spermatozoa samples will be divided into two pots to be carried out by two methods simultaneously.

RESULTS

The results of the study from the target population of all patients who carried out semen analysis tests at the Clinical Pathology Laboratory of RSIA "Restu Ibu" Sragen period from June 2020 to August 2020 obtained a total sample size of 36. The number of samples is in accordance with the calculation of the sample size needed by the researcher to obtain the appropriate data. Sampling is carried out on all semen samples who come to be examined for spermatozoa analysis, which have been educated and promised to come for the examination in sequence.

Table 2. Subject characteristic based on age

Age category	Age Range (year)	Amount	Sampling Methods
Late Adolescence	17-25	7	masturbating
Early adulthood	26-35	21	masturbating
Late adulthood	36 -45	8	masturbating
TOTAL		36	

Table 2 show characteristics of patients who are willing to be research subjects are in the age range of late adolescence to early old age. The majority of respondents are in the age range of 26-35 years or in this phase referred to as early adulthood as many as 21 people. Then at the age range of 17 to 25 years or called late adolescence as many as 7 people. Medium in the age range of 36-45 years or the phase of late adulthood as many as 8 people (Table 3-4).

Table 3. Sample characteristic based on volume and color

Semen Volume		Semen Color	
(mL)	Amount	Criteria	Amount
< 1,5	2	White Gray	17
1,5 – 5	30	Pale Yellow	10
> 5	4	Yellow	9
Total	36		36

Table 4. Sample characteristic based on liquefaction time

Liquefaction Time		Abstinence Time	
(Minutes)	Amount	(Days)	Amount
< 30	13	2 – 3	14
30 – 60	23	4 – 5	14
> 60	0	6 – 7	8
Total	36		36

The inclusion criteria table shows samples that meet the requirements to be included in the study including the volume of semen, the number of samples is 36 which will then be calculated by the researcher using manual methods and automatic methods. The aim of the researcher to get a normal sample is to make it easier to read the sperm concentration, so that the effective

time for examining each sample can be achieved.

Table 5. Count result of spermatozoa

Subject characteristic	Manual Methods	Automatic Methods
Minimum value (million cell/mm^3)	5.3	4.0
Maximum value (million cell/mm^3)	240.0	243.8
Mean (million cell/mm^3)	86.731	87.347
Standart Deviation	76.0004	76.4370
> Mean	14 (38.9 %)	22 (61.1 %)
< Mean	14 (38.9 %)	22 (61.1 %)

Table 5 contain simple descriptive test to determine the lowest and highest values, the mean value, and the standard deviation of each examination. In the manual method, the lowest examination score was 5.3 million/mm^3, the highest value was 240 million/mm^3, the average examination result was 86.731 million/mm^3 with a standard deviation of 76.0004 million/mm^3. Whereas in the automatic method, the lowest examination value was 4 million/mm^3, the highest value was 243.8 million/mm^3, the average examination result was 87.347 million/mm^3 with a standard deviation of 76.4370 million/mm^3.

This research is a type of quantitative research, the data that has been collected is analyzed statistically using the Statistical Package for the Social Science version 20 (SPSS). Before analyzing the data, a normality test was conducted to determine whether the data was normal or not. Normality test using Kolmogorow Smirnow. The data is classified as normally distributed if the p value > 0.05. Furthermore, if the data is normally distributed, then the data is t-independent test, but if the data distribution is not normal p value < 0.05 the data is analyzed by the Mann Whitney test.

The normality test of the data obtained is used to determine the distribution of the data in order to determine the statistical test used. With the Mann Whitney method, it can be seen the difference between the calculation of the sperm concentration between the manual method and the automatic method.

The Table 5 above also shows the comparison of the results against the mean on the examination of sperm concentration using the manual method. The results of the examination were below the mean value of 22 samples or as much as 61.1%, above the mean value of 14 samples or as much as 38.9%.

In the examination of sperm concentration using an automatic method. The results of the examination were below the mean value of 22 samples or as much as 61.1%, above the mean value of 14 samples or as much as 38.9%. This study shows the *p* value (significance) of the manual method with the Kolmogorov-Smirnov test of 0.002 and the Shapiro-Wilk test of 0.000. In the automatic method, the Kolmogorov-Smirnov test is 0.001 while the Shapiro-Wilk test is 0.001. Both data groups show a significance

value below 0.05, which means that both data have an abnormal distribution value. Because the two groups of data were not normally distributed, the next hypothesis test was to use the Mann Whitney method. The comparison of the calculation of sperm concentration between the manual method and the automatic method show with the Mann Whitney method, the Sig value is 0.960. Because the value of Sig> 0.05, it can be concluded that H0 is accepted and H1 is rejected, which means that there is no difference between the limitation of this study of sperm concentration between the manual method and the automatic method.

DISCUSSION

Data were obtained from patients who came to the Clinical Pathology Laboratory of RSIA "Restu Ibu" Sragen for the period June to August 2020. The sample was a population that met the criteria for semen analysis examination. The number of samples in this study were all semen samples in the Clinical Pathology Laboratory of RSIA "Restu Ibu" Taken sequentially according to the hour and day the semen sample was received by the laboratory. determine the sample size of the population using the formula Isaac & Michael according to (15) and as many as 36 samples.

Examination of sperm concentration using the manual method in this study showed results above the average by 14 and below the average by 22 samples. The data obtained from this study have been tested for normality using the Kolmogorov Smirnov and Shapiro Wilk methods. The normality test used in this study was Shapiro Wilk because the amount of data used was less than 100. The expected p value (significance) was 0.05. The p-value on the examination of sperm concentration with the manual method was 0.000 and the automatic method was 0.001. Because both groups of data have a significance value lower than the same value of 0.05, it can be said that the data distribution is not normal. The statistical test used next is the Mann-Whitney test.

The results of statistical tests using the Mann Whitney method obtained a significance value (p) of 0.960. In this study, H0 is accepted if the result is $p > 0.05$. Because the results of the study showed a p value > 0.05, H0 was accepted and H1 was rejected, meaning that there was no difference in the results of the sperm concentration examination between the manual method and the automatic method.

Basically, there are two categories of automatic sperm analyzers on the market which can be characterized by their detection technology. SQA-V GOLD is a fully automated system, which is based on the detection of electro-optical signals generated by moving sperm and interpreted by a special algorithm. This signal processing for sperm motility is combined with

spectrophotometric technology to determine sperm concentration. The Computer Assisted Sperm Analysis (CASA) is based on another principle: microscopic image capture and image processing to detect both motile and immotile spermatozoa via rapid and sequential frame acquisition (18).

This study controls for factors ranging from pre-analytic, analytic and post-analytic. In the pre-analytic factors, starting with an explanation of the criteria for the accepted sample, including abstinence, methods of dispensing and holding, and sending samples to the laboratory. In terms of analytic factors, sample processing is also carried out carefully from the stages of observing sample quality, liquefaction, and sample processing. At the post analytic stage, the researcher controls the results and records the data.

LenshookeTM SQA X1 Pro provides higher normal morphological values and average concentrations compared to the gold standard. This result occurs because LenshookeTM SQA X1 Pro uses different examination criteria, directly without staining on morphology. Although direct concentration tests were carried out in the selection of spermatozoa for Intracytoplasmic Sperm Injection (ICSI), until now the spermatozoa morphological criteria have not been applied in semen analysis (6). The results of the examination of sperm concentration in this study showed that there were no significant differences in sperm concentration between the manual method and the automatic method. This shows that the gold standard used is still used as a reference for assessing sperm concentration. And the use of automatic tools can also be considered in terms of speed, which will make it easier to work if the sample inspection is carried out in large quantities.

CONCLUSIONS

The results of the sperm concentration examination in this study showed that there was no significant difference in sperm concentration between the manual method and the automatic method.

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AUTHOR CONTRIBUTIONS

Emma Ismawatie: conceptualization, formal analysis, investigation, writing - original draft, writing - review & editing. Purwanto Adhipireno: supervision, conceptualization, methodology, writing - original draft. Seso Sulijaya Suyono: validation, formal analysis. edy purwanto: validation, reviewing. Santoso Budi: validation, reviewing. Edward Kurnia

Setiawan Limijadi: writing - review & editing, investigation.

CONFLICT OF INTEREST

There are no conflict of interest to report.

REFERENCES

1. Ring JD, Lwin AA, Köhler TS. Current medical management of endocrine-related male infertility. Asian J Androl. 2016;18(3):357–63.
2. WHO. Laboratory manual for the examination and processing of human semen. 5 ed. WHO Library. 2010.
3. Amann RP, Waberski D. Computer-assisted sperm analysis (casa): Capabilities and potential developments. Theriogenology.2014 ; 81(1)5-17.
4. Dohle G, Arver S, Bettocchi C, Jones T., Kliesch S, Punab M. Guidelines on male hypogonadism. Eur Assoc Urol. 2011;1–24.
5. Agarwal A, Henkel R, Huang CC, Lee MS. Automation of human semen analysis using a novel artificial intelligence optical microscopic technology. Andrologia.2019; 2. 1-9.
6. Bonraybo. Lenshooke XI PRO Semen Quality Analyzer. 2019; 1-4. http://bonraybio.com/en/product.php?act=view&id=8.
7. Maggavi RR, Pujari SA, Vijaykumar CN. Motility analysis with morphology: Study related to human sperm. Procedia Computer Science. 2019 ; 152. 179-85.
8. Narulita P. The Effectiveness of lenshooke tm semen quality analyzer x1 pro for human semen analysis. Fac Med Univ Airlangga, Surabaya. 2020;1(1).
9. Chaurasia A, Sinha A, Upahdyay P. Comparison of semen analysis by manual and automated method. J Pathol Nepal. 2016;6(12):990–3.
10. Dearing C, Jayasena C, Lindsay K. Can the cperm class analyser (sca) casa-Mot system for human sperm motility analysis reduce imprecision and operator subjectivity and improve semen analysis?. Human Fertility. 2014; 17(1): 37–44.
11. Baig A, Shoebuddin M, Ahmed M. Comparison of manual sperm analysis with computer-assisted sperm analysis: A comparative cross-sectional study.National Journal of Physiology, Pharmacy and Pharmacology. 2019 ; 9.1-3.
12. Syauqy A. Evaluasi kromatin sperma sebagai indikator kualitas sperma. [Evaluation of sperm chromatin as an indicator of sperm quality]. Jambi Medical Journal Jurnal Kedokteran dan Kesehatan. 2014 ; 2. 1-6.
13. Talarczyk J, Berger A, Taszarek-hauke G. Manual vs computer-assisted sperm analysis: can casa replace manual assessment of human semen in clinical practice. Ginekologia Poly.2017; 88(2);56-60.
14. Tomlinson MJ, Naeem A. CASA in the medical laboratory: CASA in diagnostic andrology and assisted conception. CSIRO Publishing. 2018 ; 30 (6)850-59.
15. Sugiyono. Metode penelitian kuantitatif kualitatif dan R&D. [Qualitative research methods and R&D]. Graha Ilmu. edisi1. Bandung, Alfabeta. 2019. 286 - 292p.
16. Singh S, Sharma S, Jain M, Chauhan R. Importance of papanicolaou staining for sperm morphologic analysis: Comparison with an automated sperm quality analyzer. American Journal of Clinical Pathology. 2011;136 (2) : 247-51.
17. Shahnaz, Ayesha. Infertility: A review on causes, treatment and management. womens health & gynecol. 2016;2(6): 35-40.
18. Park YS, Park S, Ko DS, Park DW, Seo JT, Yang KM. Observation of sperm-head vacuoles and sperm morphology under light microscope. Clinical and experimental reproductive medicine, 2014;41(3), 132–136. https://doi.org/10.5653/cerm.2014.41.3.132
19. Ariagno JI, Mendeluk GR, Furlan MJ, Sardi M, Chenlo P, Curi SM, Pugliese MN, Repetto HE, Cohen M. Computer-aided sperm analysis: a useful tool to evaluate patient's response to varicocelectomy. Asian journal of andrology, 2017;19(4), 449–452. https://doi.org/10.4103/1008-682X.173441
20. Franken DR, Oehninger S. Semen analysis and sperm function testing. Asian journal of andrology, 2012;14(1), 6–13. https://doi.org/10.1038/aja.2011.58
21. Kevin, KL, Raymond HWL, Ernest HYN, Pak CH, William SBY. Semen analysis – what a clinician should know. Continuing medical education. 2012. Avalaible online at https://www.mims-cpd.co.id/

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