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Original Research

Post-Preparation Total Motile Sperm Count in Infertile Men and The Success Rate of Intrauterine Insemination at Rumah Sakit Kasih Ibu Denpasar in 2022

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Abstract

A married couple finds themselves unable to achieve pregnancy after 12 months of regular unprotected sexual intercourse, which can be called infertility. Infertility can also occur in men. In dealing with infertility, intrauterine insemination (IUI) is a cost-effective and non-invasive Assisted Reproductive Technology (ART) procedure. The condition of male infertility can be detected through semen analysis. One of the parameters in semen analysis is the total motile sperm count (TMSC). This research seeks to establish the relationship between post-preparation TMSC and the success rate of the IUI procedure. This research adopts a descriptive-analytical design employing a cross-sectional methodology. Non-probability sampling was utilized as the sampling technique, and the data analyzed were secondary data from the medical records of male patients aged 25 to 45 years old diagnosed with infertility. The subjects had undergone IUI at Rumah Sakit Kasih Ibu Denpasar from January 1 to December 31, 2022. From the 35 obtained samples, 12 individuals (34.3%) had a post-preparation TMSC of ≥10 × 106, while 23 individuals (65.7%) had a post-preparation TMSC of <10 × 106. The success rate of the Intrauterine Insemination (IUI) procedure was 17.1%, with 4 couples (33.3%) having a post-preparation TMSC of ≥10 × 106 and 2 couples (8.7%) with a post-preparation TMSC of <10 × 106. The Fisher's Exact test carried out has obtained a result (p=0.151), while the value of the odds ratio (OR) obtained (OR=5.250. Based on statistical analysis, it can be concluded that there is no significant association between post-preparation TMSC in infertile men and the success rate of IUI procedures (p > 0.05). The odds ratio (OR) obtained is 5.250 (95% CI 0.799-34.496).

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1. Introduction

Every married couple aspires to have children, but not every pair can realise this dream. When a married couple is unable to achieve pregnancy after engaging in regular unprotected sexual intercourse for 12 months, it is termed infertility.[1] Infertility is not limited to women; it can also affect men. Male infertility is a notable global health concern, with an estimated 48 million couples and 186 million individuals worldwide facing fertility challenges.[2] The incidence of male infertility is rising in developed and developing nations.[3] Furthermore, in Indonesia, statistics indicate that male factors account for 48.4% of infertility cases.[4]

Infertility in men can be diagnosed through laboratory testing, specifically semen analysis, which examines sperm in the male seminal fluid to identify any abnormalities. This analysis includes both macroscopic and microscopic evaluations. A key factor associated with male infertility, examined in both macroscopic and microscopic assessments, is Total Motile Sperm Count (TMSC).[5]

Total Motile Sperm Count (TMSC) is the total number of moving sperm in the entire ejaculation. TMSC is obtained by multiplying the ejaculate volume by sperm concentration with the proportion of progressively motile sperm divided by 100%. Motility refers to the sperm's ability to move independently, such as the capability to move or swim. For successful sperm-egg encounters, sperm cells must move rapidly in a straight line, known as progressive motility.[6] The evidence suggests that post-preparation TMSC may offer better insights into certain fertility procedures than pre-preparation counts.[7,8]

All treatment efforts aimed at supporting fertility are intended to optimize the chances of pregnancy. Married couples with infertility often seek alternatives such as Assisted Reproductive Technology (ART) to enhance their chances of conceiving. The ART commonly utilized by the couple as an alternative to achieve pregnancy includes Intrauterine Insemination (IUI), In Vitro Fertilization (IVF), and Intracytoplasmic Sperm Injection (ICSI).[9] The ART option employed by the researchers in this study is IUI.

Intrauterine Insemination (IIU) is a non-invasive Assisted Reproductive Technology (ART) method where high-quality sperm is placed into the uterine cavity during ovulation. IIU is commonly chosen because it is simpler, more cost-effective, and less invasive than other ART procedures like IVF and ICSI. Therefore, it is a widely used ART procedure in fertility clinics and is often the first-line treatment option for patients experiencing infertility issues. Additionally, IIU has a clinical pregnancy success rate ranging from 10-20%.[10]

The correlation between clinical pregnancy and post-preparation TMSC exhibits significant variability in the literature. A retrospective study involving 526 cycles of IUI revealed that TMSC emerges as an independent factor influencing pregnancy success, particularly when TMSC is $\geq 9 \times 10^6$, associated with higher pregnancy rates.[11] However, another study assessing various semen parameters with 4,251 IUI cycles indicated that post-preparation TMSC did not hold significance in a multivariable model.[12] The high variability in study designs poses a challenge in drawing definite conclusions regarding the relationship between post-preparation TMSC in infertile men and the success of IUI. In Indonesia, discussions about this research are relatively uncommon, prompting researchers to explore the correlation between post-preparation TMSC in infertile men and the success of IUI.

Rumah Sakit Kasih Ibu is one of the hospitals that provide ART for infertility patients in Bali, especially Denpasar. The ART procedures that can be carried out at Rumah Sakit Kasih Ibu Denpasar, such as IIU and IVF, are complete. Rumah Sakit Kasih Ibu Denpasar was the researcher's choice as a research location based on considerations of various things such as being easy to reach from the researcher's residence, ease of data collection process, and considering the various limitations that researchers have regarding time, costs, etc. Based on the background mentioned previously and the lack of related research in Indonesia, especially in Bali, the author is interested in compiling research.

2. Method

This study employs a descriptive-analytical approach with a cross-sectional design, aiming to establish a correlation between the post-preparation total motile sperm count in infertile men and the success of intrauterine insemination conducted at Rumah Sakit Kasih Ibu Denpasar in 2022. The research occurs in the Andrology Laboratory at Rumah Sakit Kasih Ibu Denpasar from February to October 2023. The data comprises secondary information from patient medical records and semen analysis outcomes. Ethical approval for this research has been granted

by the Ethics Commission of the Faculty of Medicine, Udayana University, as indicated by approval letter number 354/UN14.2.2.VII.14/LT/2023.

The sampling approach employed in this research utilizes non-probability sampling through the purposive sampling method. This method involves selecting research participants based on specific criteria, including inclusion and exclusion criteria, which are considered representative of the entire population. The inclusion criteria for this study pertain to men aged 25-45 years, married and living with their wives, diagnosed with infertility, undergoing intrauterine insemination procedures between January 1 and December 31 in 2022, and having complete medical record data. On the other hand, exclusion criteria involve incomplete medical record data. Considering these inclusion and exclusion criteria, this study's total number of participants amounts to 35 samples.

Data collected and analysis were performed using SPSS for Windows 10 version 26. The analytical process included both univariate and bivariate analyses. Univariate analysis was presented in tabular format to extract frequency distribution data and proportions of independent, dependent, and confounding variables. The bivariate analysis utilized the Fisher Exact test to illustrate the relationship between post-preparation TMSC and the success rate of the IUI procedure at Rumah Sakit Kasih Ibu Denpasar in 2022, incorporating an assessment of odds ratios.

3. Result

This research yielded the following outcomes:

Table 1. Characteristics of Subjects

Variable	Frequency (n=35)	Percentage (%)
Age (years)		
25-35	20	57.1
36-45	15	42.9
Infertility Type		
Primary	18	51.4
Secondary	17	48.6
Duration of Infertility (years)		
1-4	16	45.7
>4	19	54.3
Smoking History		
Yes	14	40.0
No	21	60.0
Alcohol Consumption		
Yes	14	40.0
No	21	60.0
Origin		
Denpasar	10	28.6
Outside Denpasar	25	71.4
Pekerjaan		
Civil Servant	6	17.1
Private Employee	21	60.0
Military/Police	1	2.9
Others	7	20.0
Sperm Preparation Techniques		
Density Gradient Centrifugation	22	62.9
Simple Wash and/or Swim-Up	13	37.1
Number of IUI Cycles		
1 Cycle	33	94.3
2 Cycle	1	2.9
3 Cycle	1	2.9

In Table 1, the characteristics of the research subjects are presented based on age, type of infertility, duration of infertility, smoking history, alcohol consumption history, the origin of the research sample, the occupation of the research sample, sperm preparation technique, and the intensity of IUI (number of cycles). In the age group of 25-35 years, there were 20 subjects (57.1%), while in the age group of 36-45 years, there were 15 subjects (42.9%), with an average age of 35.1 years for the research sample. Based on the type of infertility, 18 subjects (51.4%) had primary infertility, while 17 subjects (48.6%) had secondary infertility. Regarding the duration of infertility, 16 subjects (45.7%) experienced infertility for 1-4 years, while 19 subjects (54.3%) experienced infertility for more than 4 years. In terms of smoking history, 14 subjects (40.0%) had a history of smoking, while 21 subjects (60.0%) did not have a smoking history. Additionally, based on the history of alcohol consumption, 14 subjects (40.0%) had a history of alcohol consumption, while 21 subjects (60.0%) did not have a history of alcohol consumption.

Based on occupation, there were 6 subjects (17.1%) working as civil servants, 21 subjects (60.0%) working in the private sector or as entrepreneurs, 1 subject (2.9%) working in the military/police, and 7 subjects working in other professions. Furthermore, concerning sperm preparation technique, 22 subjects (62.9%) used the density gradient centrifugation technique, while 13 subjects (37.1%) used the simple wash and/or swim-up sperm preparation technique. Lastly, based on the intensity of undergoing IUI, 33 subjects (94.3%) underwent IUI once per cycle, 1 subject (2.9%) underwent IUI twice per cycle, and 1 subject (2.9%) underwent IUI three times per cycle.

Table 2. Distribution of Samples Based on Post-Preparation TMSC

Variable	Frequency (n=35)	Percentage (%)
Post-Preparation TMSC		
$\geq 10 \times 10^6$	12	34.3
$< 10 \times 10^6$	23	65.7

Table 3. Distribution of Samples Based on the Success Rate Intrauterine Insemination

Variable	Frequency (n=35)	Percentage (%)
Success Rate IUI		
Pregnancy Positive	6	17.1
Pregnancy Negative	29	82.9

Table 4. Bivariate Analysis of Post-Preparation TMSC with the Success Rate of Intrauterine Insemination

Post-Preparation TMSC	Success Rate IUI				Total		p-value	OR (CI95%)
	Pregnancy Positive		Pregnancy Negative					
	n	%	n	%	n	%		
Post-Preparation TMSC $\geq 10 \times 10^6$	4	33.3	8	66.7	12	(100)		
Post-Preparation TMSC $< 10 \times 10^6$	2	8.7	21	91.3	23	(100)	0.151	5.250 (0.799-34.496)
Total	6	17.1	29	82.9	35	(100)		

Table 2 shows the distribution of samples with post-preparation TMSC $\geq 10 \times 10^6$, which resulted in 12 subjects (34.3%), while samples with post-preparation TMSC $< 10 \times 10^6$ obtained 23 subjects (65.7%). The data in Table 3 indicates that the majority of couples undergoing IUI procedures did not succeed or did not achieve pregnancy. Out of the total, 29 couples (82.9%) did not achieve pregnancy after the IUI procedure, while 6 couples (17.1%) successfully became pregnant after the IUI procedure. Bivariate analysis between post-preparation TMSC and the success rate of the

insemination procedure is presented in Table 4. From the 35 research samples, a higher success rate of the IIU procedure was obtained in the sample group with post-preparation TMSC ≥10 × 10^6, namely, 4 samples (33.3%), while the success rate of the IIU procedure in the sample group with post-preparation TMSC <10 × 10^6 was 2 samples (8.7%). The failure of the IIU procedure or the absence of pregnancy was also higher in the sample group with post-preparation TMSC <10 × 10^6, with 21 samples (91.3%). In comparison, the failure of the IIU procedure in the sample group with post-preparation TMSC ≥10 × 10^6 was 8 samples (66.7%).

Subsequent bivariate analysis was conducted using the Fisher's Exact Test. The p-value obtained from the Fisher's Exact test was 0.151. This indicates that there is no significant relationship between post-preparation TMSC and the success of the IIU procedure because of the value (p>0.05). This study also calculated the odds ratio (OR), obtaining a result of 5.250 (95% CI 0.799-34.496). An OR value >1 indicates that post-preparation TMSC is a factor influencing the success of the IIU procedure. However, in the confidence interval (CI 95%), there is a value of 1 in the upper and lower limit range, so in the hypothesis test, the result is that H_0 is accepted while H_1 is rejected. Therefore, in this study, it can be concluded that there is no relationship between post-preparation TMSC and the success rate of the intrauterine insemination procedure at Rumah Sakit Kasih Ibu Denpasar.

4. Discussion

Sperm, a male reproductive cell in semen fluid, moves freely to reach the ovaries and penetrate the egg during sexual intercourse.[13] The quality of sperm plays a key role in the success of the penetration and fertilization processes. Laboratory semen analysis, both macroscopic and microscopic, is used to measure sperm quality. Sperm motility is a crucial indicator related to the overall function of sperm, where sperm motility depends on structures such as the flagellum consisting of 9 pairs of spindle threads, as well as ATP energy.[14] Additionally, special attention to sperm concentration in seminal fluid and ejaculate volume is also necessary to determine the success rate of fertilization.

Total Motile Sperm Count (TMSC) involves sperm movement during ejaculation, calculated from sperm volume and concentration, with the proportion of progressively motile sperm divided by 100%.[6] As a primary determinant, TMSC significantly impacts the spontaneous pregnancy rate, exceeding conventional laboratory benchmarks and the WHO 2010 thresholds.[15] The clinically significant average TMSC in ejaculation assists in predicting successful spontaneous pregnancy rates, particularly for couples with male factor infertility.[16] TMSC can be counted either before (pre-preparation TMSC) or after semen preparation (post-preparation TMSC). Several studies suggest that assessing post-preparation TMSC may provide enhanced insights into specific fertility procedures compared to calculations performed before preparation.[7,8]

In this study, the number of samples with post-preparation TMSC ≥10 × 10^6 was 12 subjects (34.3%), while those with post-preparation TMSC <10 × 10^6 were 23 subjects (65.7%). Another retrospective review model study conducted by Muthigi *et al* in 2021 yielded results from 62,758 insemination cycles involving 37,553 patients, where 46,557 insemination cycles obtained post-preparation TMSC ≥9 × 10^6, while 16,201 insemination cycles had post-preparation TMSC <9 × 10^6.[11] Additionally, a study by Charit and Sirait, during the period of June-October 2018 at Morula IVF Jakarta, found that out of 42 samples, the highest success rate in IUI was achieved with the majority of samples having sperm counts between 4 × 10^6 to <6 × 10^6 and 6 × 10^6 to <8 × 10^6, resulting in pregnancy.[17] Therefore, these findings emphasize the importance of sperm motility in the fertilization process and the need for careful assessment of sperm motility quality to optimize the success rate of the IUI procedure.

IUI is a non-invasive ART method in which high-quality sperm is placed into the uterine cavity during ovulation. The success of the IUI procedure is assessed by the presence of clinical pregnancy, confirmed through β-hCG testing or the detection of a pregnancy sac in transvaginal ultrasound examination. The β-hCG hormone can be detected around 8-11 days after fertilization, and a β-hCG level exceeding 25 mIU/ml indicates a positive pregnancy.[18] Ultrasonography in the first trimester is generally performed to validate the presence of pregnancy and can be detected at 3-5 weeks of gestation through ultrasonography.[19] Before the IUI procedure is performed, the sperm preparation stage must be completed to separate normal sperm and obtain good motile sperm. Sperm preparation

involves simple wash, swim-up, and density gradient centrifugation. In the simple wash method, the semen sample is liquefied in a medium and then centrifuged to separate motile sperm from other components.[20] The swim-up technique is suitable for semen with high sperm concentration, and the sperm will move by allowing it to swim against gravity (towards the upper layer of the sperm medium) after being separated from the seminal fluid.[21] Lastly, the density gradient centrifugation (DGC) technique uses a reaction tube filled with several liquid layers of different densities and often produces high-quality sperm for fertilization compared to the previous techniques.[22]

The results of this study indicate a low occurrence of positive pregnancies, with 6 couples (17.1%), while 29 couples (82.9%) did not achieve pregnancy. This finding aligns with the study conducted by Salim *et al* at Rumah Sakit Kasih Ibu Denpasar in 2021, which reported a similar percentage of around 6 couples (17.1%) out of a total of 35 samples undergoing IUI from January to December 2020.[23] Other studies indicate a significant success rate in procedures, as evidenced by research conducted by Charit and Sirait during the period of June-October 2018 at Morula IVF Jakarta, Indonesia. The study found that out of 412 couples undergoing IUI, 42 couples (10%) achieved a positive pregnancy, while 379 couples (90%) did not achieve pregnancy.[17] This indicates that the success rate of the IUI procedure at Rumah Sakit Kasih Ibu Denpasar is quite worthy of being a reference related to the hospital's track record with a good IUI success rate in Indonesia, especially in Bali.

The Fisher's Exact test conducted aimed to assess the relationship between independent and dependent variables yielded a p-value of 0.151, with $p>0.05$. The study also calculated the odds ratio (OR), resulting in a value of 5.250 (95% CI 0.799-34.496). An OR value >1 indicates that post-preparation TMSC is a factor influencing the success of the IUI procedure. However, within the 95% confidence interval (CI), there is a value of 1 in the upper and lower bounds range. Therefore, it can be concluded from this study that there is no relationship between post-preparation TMSC and the success rate of intrauterine insemination. This could be attributed to the sperm preparation process preceding IUI, ensuring that only sperm of high quality was utilized in the investigation.

The correlation between clinical pregnancy and post-preparation TMSC varies significantly in the literature. A retrospective study conducted by Muthigi *et al*, involving 526 IUI cycles, revealed that TMSC emerged as an independent factor influencing pregnancy success, especially when the TMSC count was ≥ 9 million sperm, associated with a higher pregnancy rate. This study found a significant relationship, as demonstrated by Generalized Estimating Equations (GEE) analysis, indicating that high TMSC significantly predicts pregnancy success in IUI cycles. These results highlight the important predictive value of TMSC in determining IUI success.[11] However, another study conducted by Lemmens *et al*, evaluating various semen parameters with 4,251 IUI cycles, indicated that post-preparation TMSC did not have significance in the multivariable model. In this study, no significant relationship was found between TMSC and the likelihood of achieving pregnancy, especially after the first IUI cycle.[12]

The success of the IUI procedure is not determined by a single factor but can be impacted by a combination of internal and external factors and confounding factors.[23,24] Internal factors include the age of both men and women, medical history, and anatomical abnormalities. External factors encompass elements such as alcohol consumption, smoking habits, and workplace conditions that may expose individuals to metal substances. Confounding factors involve the role of healthcare providers (gynaecologists) and factors related to women. This investigation primarily concentrates on the association between post-preparation TMSC in infertile men and the success rate of IUI, considering it as one of the semen analysis parameters linked to IUI success. Due to the study's limitations, internal, external, and confounding factors were not incorporated into the analysis. These limitations and other factors influencing IUI success have not been thoroughly examined, warranting further research to explore these aspects.

5. Conclusion

There is no statistically significant association between post-preparation TMSC in infertile men and the success rate of IUI, as indicated by a p-value of 0.151 because the p-value is >0.05. The calculated odds ratio (OR) reveals a value of 5.250 (95% CI 0.799-34.496). An OR value >1 suggests that TMSC is a factor influencing the success of the IUI procedure. However, the confidence interval (CI 95%) includes the value of 1 within the upper and lower limits. Consequently, the hypothesis test results in the acceptance of H_0 and the rejection of H_1 indicate that there is no relationship between

post-preparation TMSC and the success rate of intrauterine insemination procedures at Rumah Sakit Kasih Ibu Denpasar.

Further research with a larger sample size and a more in-depth analysis of internal, external, and confounding risk factors is needed. Examining other factors such as lifestyle, health history, patient genetic factors, provider factors, and factors related to women that may affect the success of IUI can provide further insights. This research aims to enhance public understanding, particularly among couples, urging them to prioritize reproductive health and organ function before undergoing IUI procedures. Moreover, it encourages a transition toward a healthier lifestyle.

Author's Contribution

All authors played a role in shaping the final manuscript. The first author gathered and processed data, analyzed and interpreted data, drafted the manuscript, and created figures. The second and third authors contributed to outlining the main conceptual ideas of the research and providing critical revisions to the article.

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Conflict of Interest

The authors assert that there are no conflicts of interest concerning this research.

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Ethics Approval

Ethical clearance for this research was obtained from Ethics Commission of the Faculty of Medicine, Udayana University, as indicated by approval letter number 354/UN14.2.2.VII.14/LT/2023.

References

1. World Health Organization. Infertility [Internet]. *WHO*. 2023 [cited 2024 Jan 1]. Available from: https://www.who.int/publications/i/item/9789240068315
2. Ombelet W. WHO Fact Sheet on Infertility Gives Hope to Millions of Infertile Couples Worldwide. *Facts, views Vis ObGyn*. 2020;12(4). [Pubmed]
3. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A Unique View on Male Infertility Around the Globe. *Reprod Biol Endocrinol*. 2015;13(1). [Pubmed]
4. Birowo P, Jelita NK, Sari P, Rasyid N. Hubungan Kadar FSH dengan Gambaran Spermatogenik pada Pasien Azoospermia Berdasarkan Kriteria Johnson. *eJournal Kedokteran Indonesia*. 2018;5(3). [Journal]
5. Persandi. *Standarisari Analisa Semen Nasional*. 2016. Available from:
6. Hajder M, Hajder E, Husic A. The Effects of Total Motile Sperm Count on Spontaneous Pregnancy Rate and Pregnancy After IUI Treatment in Couples with Male Factor and Unexplained Infertility. *Med Arch (Sarajevo, Bosnia Herzegovina)*. 2016 Feb 1;70(1):39–43. [Pubmed]
7. Yavuzcan A, Yurtçu E, Keyif B. Is There Any Effect of Change in Pre-Wash and Post-Wash Semen Parameters on the Success of Intrauterine Insemination? 2024;4–15. [Pubmed]
8. Qureshi SM, Kafeel S, Bibi R, Mohmand J. Post-wash Total Motile Sperm Count A Useful Predictor in the Decision to Perform IVF/ICSI in Patients with Non-Male Factor Infertility. *J Shifa Tameer-e-Millat Univ*. 2020;3(2). [Journal]
9. Hododjojo E, Queljoe E De, Tendean OS. Indikasi Inseminasi Intrauterine Akibat Faktor Pria. *J Kedokt Komunitas Dan Trop*. 2015;3(3). [Journal]
10. Allahbadia GN. Intrauterine Insemination: Fundamentals Revisited. Vol. 67, Journal of Obstetrics and *Gynecology of India*. 2017. [Pubmed]
11. Muthigi A, Jahandideh S, Bishop LA, Naeemi FK, Shipley SK, O'Brien JE, et al. Clarifying the Relationship between Total Motile Sperm Counts and Intrauterine Insemination Pregnancy Rates. In: Fertility and Sterility. *Elsevier Inc*. 2021. [Pubmed]
12. Lemmens L, Kos S, Beijer C, Brinkman JW, van der Horst FAL, van den Hoven L, et al. Predictive Value of Sperm Morphology and Progressively Motile Sperm Count for Pregnancy Outcomes in Intrauterine Insemination. *Fertil Steril*.

2016;105(6). [Pubmed]
13. Carlson BM. Chapter 14 - The Reproductive System. In: The Human Body. Academic Press; 2019. p. 373–96.
14. Linck RW, Chemes H, Albertini DF. The Axoneme: The Propulsive Engine of Spermatozoa and Cilia and Associated Ciliopathies Leading to Infertility. Vol. 33, *Journal of Assisted Reproduction and Genetics*. 2016. [Pubmed]
15. Hamilton JAM, Cissen M, Brandes M, Smeenk JMJ, De Bruin JP, Kremer JAM, et al. Total Motile Sperm Count: A Better Indicator for The Severity of Male Factor Infertility Than the WHO Sperm Classification System. *Hum Repro*d. 2015 May 1;30(5):1110–21. [Pubmed]
16. Leslie SW, Soon-Sutton TL KM. Male Infertility [Internet]. StatPearls [Internet]. *StatPearls Publishing*; 2023 Jan-; 2023 [cited 2023 Mar 3]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK562258/
17. Charit AB, Sirait BI. Jumlah Sperma Motil yang Memberikan Keberhasilan Tertinggi pada inseminasi Intra Uterin di Morula IVF Jakarta Periode Juni-Oktober 2018. Vol. I, *Jurnal Ilmiah WDYA Kesehatan dan Lingkungan*. 2020. p. 221–8. [Journal]
18. Gnoth C, Johnson S. Strips of Hope: Accuracy of Home Pregnancy Tests and New Developments. *Geburtshilfe Frauenheilkd*. 2014;74(7). [Pubmed]
19. Murugan VA, Murphy BO, Dupuis C, Goldstein A, Kim YH. Role of Ultrasound in the Evaluation of First-Trimester Pregnancies in the Acute Setting. *Ultrasonography*. 2020;39(2). [Pubmed]
20. Nordhoff V, Kliesch S. Sperm preparation for therapeutic IVF. In: *Principles of IVF Laboratory Practice: Optimizing Performance and Outcome*s. 2017.
21. Fácio CL, Previato LF, Machado-Paula LA, Matheus PCS, Araújo Filho E. Comparison of Two Sperm Processing Techniques for Low Complexity Assisted Fertilization: Sperm Washing Followed by Swim-Up and Discontinuous Density Gradient Centrifugation. *J Bras Reprod Assist*. 2016;20(4). [Pubmed]
22. Ali AH, Ajina T, Ali M Ben, Mehdi M. Efficacy of density gradient centrifugation technique (DGC) in enhancing sperm cell DNA quality for assisted reproductive technique. *Middle East Fertil Soc J*. 2022;27(1). [Springer]
23. Salim A, Kurniawan Y, Pramesemara IGN. Relationship Between Smoker Male Partner and Intrauterine Insemination Success in Kasih Ibu General Hospital Denpasar. *Indones Androl Biomed J*. 2021;2(2). [Journal]
24. Starosta A, Gordon CE, Hornstein MD. Predictive factors for intrauterine insemination outcomes: a review. *Fertil Res Pract.* 2020;6(1). [Pubmed]