



Original Research

Leukospermia in Infertile Men and Successful Intrauterine Insemination at Rumah Sakit Ibu dan Anak Puri Bunda Denpasar

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Abstract

After a year of regular sexual activity without the use of contraceptives, infertility is a condition that culminates in the lack of pregnancy. The procedure known as intrauterine insemination (IUI) is frequently used in fertility clinics and is typically the first course of action for patients who have been diagnosed with infertility disorders, infertility brought on by cervical factors, infertility brought on by male factors, or infertility for unknown reasons. IUI is another easy, affordable, and effective method of treating infertility. Leukospermia is one of the variables that can affect how well an IUI procedure goes. A cross-sectional design and medical records of infertile patients, aged 25 to 45, who underwent IUI treatments at the RSIA Puri Bunda Denpasar from January to June 2022 were used in this analytical study. Purposive sampling was utilized in this study, and it was chosen based on inclusion and exclusion criteria. 53 samples were acquired for the investigation. Thirteen (24.5%) of the 53 samples had leukospermia, while 40 (75.5%) did not (of the 53 total samples). In this study, 17 patients (32.1%) who had IUI successfully, including 14 non-leukospermic subjects (35.0%) and 3 leukospermic subjects (23.1%). According to the Fisher's Exact test results, which showed a p value of 0.511, leukospermia and the success of IUI do not have a significant link. The odds ratio (OR) calculation yielded a result of 1.759 (CI95% 0,423-7,610).

1. Introduction

Most married couples undoubtedly sincerely desire offspring as successors. As for traditions for producing children, many parts of the world have them as well. For married couples, infertility or obstacles to having children are major issues. Since there is still a lack of understanding of infertility in society, it is important to promote awareness of the condition so that infertile couples won't feel ashamed or scared to see a doctor.

Infertility is a condition that does not result in pregnancy after a year of regular sexual activity without the use of contraception. The wife is frequently blamed by society for infertility. Up to 25% of couples or married couples struggle with infertility, and studies reveal that 64% of the time, the wife is to blame and 36% of the time, the husband.¹

The incidence of infertility caused by female factors as well as male factors varies according to the age of the couple trying to conceive. Male infertility is defined as the absence of pregnancy after one year of unprotected sexual intercourse in conjunction with aberrant sperm function, abnormal sperm analysis, or a disruption in the mechanism that delivers sperm to the ejaculatory tract. It is typically brought on by a condition that either affects the generation of sperm or the transportation of sperm. The doctor will be able to determine the root of the issue by conducting a series of diagnostic tests.²

The prevalence of male infertility is increasing, especially in nations that are still in the development stage. One of these examples is found in Poland, where the pattern of infertility reveals a rise in the rate of male factors contributing to infertility (57.8%), as well as a fall in the rate of female factors contributing to infertility (7.72%). 76% of the total occurrences were attributable to the male factor, while only 26% were attributable to the female component. Infertility rates among men are also rising, according to statistics collected in the UK. This situation may have arisen as a consequence of an increase in the number of publications that discuss the availability of therapy, which in turn has led to an increase in the desire of couples to seek guidance and treatment.³ In Indonesia, male infertility reaches a fairly high percentage, which is between 40 and 60% of the total cases.⁴

It is estimated that around half of all cases of male infertility may be traced back to male variables, specifically a low proportion of motile sperm and a low sperm count. It is possible to obtain highly helpful information on the reproductive status of a young man through the use

of conventional methods of sperm analysis. Up until this point, the characteristics of volume, color, viscosity, pH, concentration, motility, and morphology have been considered in the evaluation of semen. According to the results of this study, it is possible to determine whether or not a man is fertile. Semen analysis is an important examination in determining a man is fertile or infertile. The standard examination is to evaluate sperm concentration, motility, and morphology.⁵

Male patients with infertility will, of course, have trouble having children for their families, which will lead them to search for other alternatives to getting pregnant, such as assisted reproductive technology (ART). ART refers to procedures and therapies intended to induce pregnancy. For those who have completed infertility therapy but have not yet conceived, this procedure may be an option.⁶

In general, this assisted reproductive procedure involves removing the egg from the woman's ovary, combining the egg with sperm in a laboratory, and placing the embryo back into the woman's body. A cohort study over 10 years between 1998 and 2007 resulted in live births of 52% after 3 cycles (median number of cycles per patient), 72% after 6 cycles and 85% after 12 cycles. Examples of assisted reproductive technologies that are often the choice are In Vitro Fertilization (IVF) and Intrauterine Insemination (IUI).⁶

There are several main factors that can be associated with male infertility, one of which is leukospermia. Leukocytes are the active unit of the human body's defense system. Leukocytes in semen have an important role in the immune system and are also abnormal sperm phagocytosis. In semen analysis, the number of leukocytes is one of the parameters that must be examined. The presence of leukocytes in semen can provide quite useful and meaningful information in the examination of semen analysis. Microscopically, leukocytes are non-sperm cellular elements and an increase in the number of leukocytes of more than 1 million per milliliter of semen is indicative of a state of inflammation and infection.⁵

Leukospermia significantly causes infertility in men. In infertile men, the prevalence of leukospermia is 20%.⁷ The prevalence of leukospermia is very often found in infertile men and in inflammatory conditions that are highly correlated with semen quality, namely impaired sperm motility and in vitro fertilization capacity, and results in decreased sperm survival in the female reproductive tract.⁸

Patients with infertility due to leukospermia had a lower pregnancy rate than those with

infertility not due to leukospermia, according to research on the use of intrauterine insemination (IU) so far. Infertility patients with leukospermia experience a pregnancy rate of 30% after the IU procedure, compared to a pregnancy rate of 39% in infertility patients without leukospermia.

Researchers are interested in examining the connection between leukospermia and the success rate of IUI in Denpasar based on the description provided above. Due to a dearth of related studies and literature, the researchers picked Denpasar.

Researchers opted to perform their study at the Rumah Sakit Ibu dan Anak (RSIA) Denpasar because it is one of the hospitals in the city with a unique ART program, has a successful IUI rate of 13% in 2021, and has data on patients with leukospermia. For couples who intend to perform IUI in the future, it is hoped that this research would be helpful.

2. Method

This investigation was conducted between January and June of 2022 at the RSIA Puri Bunda Denpasar. With letter number 347/UN14.2.2.VII.14/LT/2022, Komisi Etik Fakultas Kedokteran Universitas Udayana has granted ethical approval. The form of research to be conducted was a cross-sectional retrospective analysis. Patients diagnosed with primary or

secondary infertility, undergoing IUI procedures between January and June 2022, and possessing a complete medical record met the inclusion criteria for this study. In this investigation, the exclusion criteria were incomplete medical record data. The sampling method employed was purposeful sampling. Using the Lemeshow formula, the minimum number of samples required for this investigation was calculated to be 32. Existing data from medical records that match the inclusion and exclusion criteria, Statistical Package for the Social Sciences (SPSS) software, Microsoft Word, and Microsoft Excel will serve as research instruments.

The analysis of the data included univariate and bivariate analysis. Univariate analysis to obtain the distribution of frequencies and proportions of the independent variables, dependent variables, and controlled variables, presented in tables and numbers. Using Fisher's Exact test, examines the relationship between the dependent variable, efficacy of the IUI procedure, and the independent variable, leukospermia. The Fisher's Exact test is used when the conditions for the Chi-Square test are not fulfilled, namely the presence of a cell with an observed value of zero and cells with an expected value greater than 5.⁹ This bivariate analysis is followed by the calculation of the Odds Ratio (OR) to determine the ratio of the exposed group's possible outcomes to those of the non-exposed group.¹⁰

3. Results

Table 1. Distribution of Sample Characteristics Based on Age, Diagnosis, and Duration of Infertility

Variable	Frequency (n = 53)	Percentase (%)
Age		
25-35 years	32	60,4
36-45 years	21	39,6
Duration of Marriage		
1-4 years	35	66,0
>4 years	18	34,0
Types of Infertility		
Primary Infertility	27	50,9
Secondary Infertility	26	49,1
Smoking history		
Yes	14	26,4
No	39	73,6
Alcohol history		
Yes	1	1,9
No	52	98,1
Origin		
Denpasar	18	33,9
Outside Denpasar	35	66,1
Occupation		
Private Employees	32	60,3
Government Employees	17	32,1

Unemployment	4	7,6
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Table 2. Sample Distribution Based on History of Leukospermia

Variable	Frequency (n = 53)	Persentase (%)
Leukospermia History		
Yes	13	24,5
No	40	75,5

Table 3. IUI Success Score Distribution

Variable	Frequency (n = 53)	Persentase (%)
IUI Results		
Succeed (Pregnant)	17	32,1
Not Successful (Not Pregnant)	36	67,9

Table 4. The Relationship Between Leukospermia in Infertile Men and Successful Intrauterine Insemination at Rumah Sakit Ibu dan Anak Puri Bunda Denpasar

Leukospermia History	IIU				Total	p	OR	
	Succeed		Not Successful					
	n	%	n	%				
Leukospermia	3	(23,1)	10	(76,9)	13	(100,0)	0,511	1,795
Non-leukospermia	14	(35,0)	26	(65,5)	40	(100,0)		
Total	17	(32,1)	36	(67,9)	53	(100,0)		

The study used the medical records of patients at RSIA Puri Bunda Denpasar from January to June 2022 to determine its results. The results showed that the research sample consisted of 53 male patients. This study's sample consisted of male infertile patients aged 25 to 45 who were married and undergoing IIU.

According to Table 1, there are two age groups: those between the ages of 25 and 35, and those between 36 and 45. There were 32 persons in the sample data for patients with an age range of 25-35 years, making up 60.4% of the total, while there were 21 people in the age range of 36-45 years, making up 39.6% of the total, and the average age of the patients was 34.6 years. There were 27 patients who were diagnosed with primary infertility, accounting for 50.9% of the total, and there were 26 patients who were diagnosed with secondary infertility, accounting for 59.1% of the total. The duration of a patient's infertility ranged from approximately one to four years in approximately sixty-six percent of cases, while

thirty-four percent of cases had a duration of more than four years.

History of smokers in the sample showed that there were 14 people (26.4%), and 39 people who were not smokers (73.6%), whereas patients who had a history of consuming alcohol were only one person (1.9%), and 52 people (98.1%) who did not have a history of alcohol consumption. Patients were located living outside of Denpasar in greater numbers, as many as 35 persons (66.1%), but patients living in Denpasar were only identified in the number of 18 people (33.9%). There were 32 people working in the private sector, which made up 60.3% of the sample, followed by 17 people working in the public sector, which made up 32.1%.

According to Table 2, 24.5% of the patients had leukospermia, while 75.5% of the patients did not have leukospermia. There were 40 patients who did not have leukospermia. Table 3 shows that 17 people managed to achieve pregnancy (32.1%)

after carrying out IUI and there were 36 other people who failed to achieve pregnancy (67.9%).

The relationship between leukospermia in infertile men and IUI success is as shown in Table 4. Of the 53 samples collected, the highest success rate was found in non-leukospermic patients with a sample of 14 people (35.0%), while the IUI failure rate in patients non-leukospermic were 26 patients (65.5%). Leukospermic patients showed a lower IUI success rate (3 people (23.1%)) compared to IUI failure, which was 10 people (76.9%).

Leukospermia and IUI success were not significantly correlated, according to the Fisher's Exact test, which had a p value of 0.511 (p value > 0.05). The OR, which is equal to 1.795 (95% CI 0.423-7.610) in this study, has also been calculated.

4. Discussion

The results of the research indicated that the total number of samples comprised 53 male patients with reports of infertility who underwent IUI testing at RSIA Puri Bunda Denpasar.

Because there were so few people in the sample, the researchers made the decision to exclude patients older than 45 years old to eliminate the possibility of age-related study bias. The process of aging in humans may be divided into three subclinical phases: the subclinical phase, which occurs between the ages of 25 and 35, the transitional phase, which occurs between the ages of 35 and 45, and the clinical phase, which occurs after the age of 45. It should be noted that in the clinical phase, men will experience a decrease in the function of the sexual organs, which can affect the formation of sperm cells.¹¹

Between the months of January and June 2022, the vast majority of male patients who undergo IUI treatment at RSIA Puri Bunda Denpasar are between the ages of 25 and 35, with the average patient being 34.6 years old. These findings are comparable to those found in a study that was carried out in 2018 by Rachmiawaty et al. at the Aster clinic at the Hasan Sadikin Hospital in Bandung with a total of 98 couples who underwent the IUI treatment. According to the findings of the study, the typical age of the male partner was determined to be 36.76 years old.¹²

When sexual activity without the use of contraception continues for a year without a pregnancy, the condition is known as male infertility.² Primary infertility and secondary infertility are the two distinct subtypes of infertility that can affect a couple's ability to have children. In this particular research, there were 27 patients who

had primary infertility (50.9%), and there were 26 patients who had secondary infertility (49.1%). The same findings were shown in a study that was carried out in 2021 at RSIA Puri Bunda Denpasar by Madeleine and her colleagues. The research involved 31 patients who were being treated for infertility, and it was discovered that there were 9 patients suffering from primary infertility (29.0%), and 22 patients suffering from secondary infertility (71.0%).¹³

This research also shows how important reproductive health is in cases of infertility. As for this study, it was found that 35 patients (66.0%) experienced infertility between 1-4 years old compared to >4 years, namely 18 people (34.0%). This is based on previous studies which showed a significant effect between the duration of infertility and the success of IUI seen in the duration of infertility <5 years and >5 years.¹⁴

In the condition known as leukospermia, the amount of leukocytes in the ejaculate is higher than one million per milliliter.¹⁵ It has been discovered that infertile men with leukospermia have decreased sperm motility, increased sperm DNA damage, and impaired fertilization capacity, influencing IUI results.¹⁶ IUI is one of the treatments that is commonly utilized in fertility clinics and is typically the initial course of treatment that is recommended to individuals who are struggling with infertility issues. Beta-hCG levels are one way that the effectiveness of IUI can be evaluated. Women who were pregnant and had a positive pregnancy test also had Beta-hCG levels that were more than 25 mIU/ml.¹⁷ In this study, there were 17 pregnancy rates (32.1%) and leukospermia patients had at least IUI success, namely 3 people. (17.6%) compared to non-leukospermic patients as many as 14 people (82.4%) and the rest, namely IUI failure in leukospermic patients as many as 10 people (27.8%) and 26 people (72.2%) in non-leukospermic patients. This can also be seen in the research by Widyastuti et al. in 2018 out of a total of 46 patients there were 18 patients (39.0%) non-leukospermic and 14 patients (30.0%) leukospermic who successfully underwent the IUI procedure. From these data it can also be said that leukospermic patients experience less success than non-leukospermic patients.¹⁸

Infertility can be affected by a history of smoking.¹⁹ It has been shown that smoking has a negative impact on infertility. According to the results of a study that was carried out by Ramlau-Hansen et al. between the years 1987 and 2004, a cross sectional examination of the data showed that smokers had decreased sperm volume, sperm count, and percentage of motile sperm when

compared to men who did not smoke. This was the case for 2542 males who underwent sperm analysis.²⁰ In this study, there were a total of 39 persons, 14 of them had a history of smoking (26.4%), and the remaining 39 (73.6%), were non-smokers.

Infertility can also be affected by a history of alcohol consumption in the patient. Several studies have shown that long-term heavy drinking can result in testicular atrophy, lower testosterone levels, and decreased sperm production, all of which can affect the ability to conceive.²¹ In studies that have been carried out, only 1 patient (1.9%) had a history of alcohol consumption, whereas in patients without a history of alcohol consumption, there were 52 people (98.1%).

Male infertility is a condition that occurs when a pregnancy fails to occur after one year of sexual intercourse without the use of contraception. This condition is also accompanied by abnormalities in the analysis of the sperm and the sperm's ability to function.² IUI is the type of assisted reproductive technology that people who are looking for other ways to have children opt for the most frequently when they are considering their options. IUI is carried out by injecting the sperm of the man into the uterus of the female during the period that she is ovulating. The success of IUI is not only determined by elements pertaining to the woman, but it can also be determined by the quality of the sperm that is provided by the male.²²

Leukospermia is one of the primary causes that can be related with male infertility. There are also several other important variables that can be associated with male infertility. The generation of reactive oxygen species (ROS) by neutrophils is the first step in the development of leukospermia. This ROS then interferes with the formation of mitochondrial DNA and intracellular ATP, which in turn damages sperm activity, motility, and function and leads to leukospermia.²³

As for the aim of this study, its objective is to determine whether or not there is a connection between leukospermia and the success of IUI. Leukospermia is a condition that can be described when the quantity of leukocytes in the sperm is greater than 1 million/ml of ejaculate and occurs when there is an increase in the amount of leukocytes in the ejaculate.¹⁵

According to the results of the research that has been carried out, it has been found that 3 leukospermic patients had IUI success, which accounts for 6% of the total, while the number of non-leukospermic patients who had IUI success was 13 which accounts for 26% of the total. The number of leukospermic patients who failed to achieve IUI success was more than those who

managed to achieve IUI success, namely as many as 10 people (20.0%), and in non-leukospermic patients who experienced IUI failure as many as 24 people (48.0%).

The results of the Fisher Exact test which was carried out as a statistical test obtained a p value of 0.511 which can be concluded that H₁ is rejected, which means there is no significant relationship between leukospermia and IUI success.

As for other research conducted by Widyastuti et al. in 2018. A total of 46 participants were collected for the study, including 18 patients who did not have leukospermia (39.0%) and 14 patients who did have leukospermia (30.0%) and who had successfully undergone the IUI process. In this study, bivariate analysis was obtained with a p value <0.05, which indicates that there is a substantial correlation between the presence of leukospermia and the success of IUI.¹⁷

Another journal by Al-Dujaily et al. in 2015 a total of 70 patients consisting of 5 normozoospermic (7.1%), 2 leukospermic (2.8%), 1 teratozoospermic (1.4%), and 1 leukospermic and teratorzoospermic managed to achieve IUI success (1.4%) and bivariate analysis was carried out with the result p <0.012 which means that there is a significant relationship between leukospermia and IUI success.²⁴

A study of other types of ART using ICSI was carried out by Cavagna et al. in 2012. A study of 50 male patients included Group 1, namely 25 leukospermic patients and Group 2, namely 25 non-leukospermic patients. The pregnancy success rate was obtained by 6 people (24%) in Group 1 and 6 people (24%) in Group 2. The test results showed a p value <0.05 which stated that there was a significant relationship between leukospermia and ICSI success.²⁵

Journals with other types of ART were also carried out by Ricci et al. in 2015 using ART in the form of IVF and ICSI. This study was conducted on 164 patients, namely IVF was performed by 59 patients consisting of 10 leukospermic patients and 49 non-leukospermic patients, while ICSI was performed by 27 leukospermic patients and 78 non-leukospermic patients. The clinical pregnancy rate per aspiration cycle was obtained, namely 25% in leukospermic patients and 14% in non-leukospermic patients. The test results obtained p = 0.13 (p > 0.05) which indicated that there was no significant relationship in this study.²⁶

The success of IUI is not only determined based on one factor, such as leukospermia, because it can be determined by many other factors. become a variable that can affect the success of IUI. This study also had no results on the influence of sperm analysis on patients. Infertile men who experience

leukospermia are found to experience decreased sperm motility, increase sperm DNA damage and affect fertilization ability.¹⁶ The results of semen analysis can provide an overview of matters that can affect the success of IUI. Further research is needed about this in future research.

5. Conclusion

There was no significant relationship between leukospermia in infertile patients and IUI success with $p = 0.511$. The IUI success rate at RSIA Puri Bunda Denpasar from January to June 2022 shows a pregnancy rate of 32.1%. The incidence of leukospermia in patients undergoing IUI at RSIA Puri Bunda Denpasar was 13 people. Calculation of the OR value obtained a number of 1.795 (95% CI 0.423-7.610).

There is a need for additional research on other risk factors that can affect the success of IUI, such as obesity and the influence of other sperm analysis results that were not detected in this study. These factors were not investigated in this particular study. It is hoped that it will also be able to be considered in the management of infertility and that it will be able to educate the public, especially men, to be watchful against leukospermia.

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Author's Contribution

All authors have contributed to the final manuscript. The contribution of each author as follow: collected the data, drafted the manuscript and designed the figures, devised the main conceptual ideas and critical revision of the article. All authors discussed the results and contributed to the final manuscript.

Conflict Of Interest

The Authors State There Is No Conflict Of interest.

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References

1. Munir. *Infertilitas. Direktorat Jenderal Pelayanan Kesehatan*, 2019.
2. Gibson, Hammoud. *Clinical Reproductive Medicine and Surgery*. Springer International Publishing AG.
3. Kupis Ł, Dobroński PA, Radziszewski P. Varicocele as A Source of Male Infertility - Current Treatment Techniques. *Cent Eur J Urol* 2015; 68: 365–370.
4. Yuwono. *Infertilitas pada Pria di Indonesia Merupakan Masalah yang Perlu Perhatian*. 2017.
5. Hasibuan, Hamid MD. *Hubungan Leukositospermia dengan Pertumbuhan Koloni Bakteri pada Kultur Cairan Semen Pria dari Pasangan Infertil Di RSUP H Adam Malik Medan*. Universitas Sumatera Utara, 2015.
6. Hododjojo E, Queljoe E De, Tendean OS. Indikasi Inseminasi Intrauterine Akibat Faktor Pria. *J Kedokt Komunitas dan Trop*; III.
7. Agarwal A, Mulgund A, Alshahrani S, et al. Reactive Oxygen Species and Sperm DNA Damage in Infertile Men Presenting with Low Level Leukocytospermia. *Reprod Biol Endocrinol* 2014; 12: 126.
8. Sandoval JS, Raburn D, Muasher S. Leukocytospermia: Overview of Diagnosis, Implications, and Management of A Controversial Finding. *Middle East Fertil Soc J* 2013; 18: 129–134.
9. Kim H-Y. Statistical Notes for Clinical Researchers: Chi-Squared Test and Fisher's Exact Test. *Restor Dent Endod* 2017; 42: 152–155.
10. Furcada JM, Patino CM, Ferreira JC. Estimating risk in Clinical Studies: Odds Ratio and Risk Ratio. *J Bras Pneumol publicacao Of da Soc Bras Pneumol e Tisiologia* 2020; 46: e20200137.
11. Siswanto FM, Pangkahila A. The Balancing of Physical Fitness Increase the Activity of Endogenous Stem Cell for Anti-Aging. *Sport Fit J* 2014; 2: 1–9.
12. Rachmiawaty A, Djuwantono T, Sasotya RMS. Analisis Faktor yang Memengaruhi Keberhasilan Kehamilan pada Inseminasi Intrauterin. *Indones J Obstet Gynecol Sci*; 1. Epub ahead of print 2018. DOI: <http://dx.doi.org/10.24198/obgynia.v1n1.42>.
13. Madeleine A, Yukhi Kurniawan, I Gusti Ngurah Pramesemara. Relationship Between the Age of Female Partner and the Intrauterine Insemination Success in Rumah Sakit Ibu dan Anak Puri Bunda Denpasar. *Indones Androl Biomed J* 2022; 3: 7–12.
14. Ahmed K. Allow, A SSMA, Maryam B, et al.

- The Relationship between Duration of Infertility and Intrauterine Insemination: A Multi-Centers Study. *J Clin Dev Biol*; 1. Epub ahead of print 2016. DOI: 10.21767/2472-1964.100013.
15. Brunner RJ, Demeter JH, Sindhvani P. Review of Guidelines for the Evaluation and Treatment of Leukocytospermia in Male Infertility. *World J Mens Health* 2019; 37: 128–137.
 16. Moubasher A, Sayed H, Mosaad E, et al. Impact of Leukocytospermia on Sperm Dynamic Motility Parameters, DNA and Chromosomal Integrity. *Cent Eur J Urol* 2018; 71: 470–475.
 17. Gnoth C, Johnson S. Strips of Hope: Accuracy of Home Pregnancy Tests and New Developments. *Geburtshilfe Frauenheilkd* 2014; 74: 661–669.
 18. Rini Widyastuti, Julius Pangayoman, Aida Riyanti, Alkaustariyah Lubis MRAAS. Pregnancy Rate after Intrauterine Insemination with the Presence or Absence of Leukocytospermia in Sperms Prepared using Density Gradient Method. *Int J Integr Heal Sci*; 6. Epub ahead of print 2018. DOI: 10.15850/ijih.v6n2.1318.
 19. Cavallini G, Beretta G. Clinical Management of Male Infertility, Springer International Publishing. Springer Cham, 2014.
 20. Kovac JR, Khanna A, Lipshultz LI. The Effects of Cigarette Smoking on Male Fertility. *Postgrad Med* 2015; 127: 338–341.
 21. Van Heertum K, Rossi B. Alcohol and Fertility: How Much is Too Much? *Fertil Res Pract* 2017; 3: 10.
 22. Allahbadia GN. Intrauterine Insemination: Fundamentals Revisited. *Journal of obstetrics and gynaecology of India* 2017; 67: 385–392.
 23. Pentyala S, Lee J, Annam S, et al. Current Perspectives on Pyospermia: A review. *Asian J Androl* 2007; 9: 593–600.
 24. Al-Dujaily SS, H.Al-Jnabi M, Jasim SN. The Influence of Leukocytospermia and Teratozoospermia in IUI Outcome. *Int J Adv Res* 2015; 255–265.
 25. Cavagna M, Oliveira JBA, Petersen CG, et al. The Influence of Leukocytospermia on The Outcomes of Assisted Reproductive Technology. *Reprod Biol Endocrinol* 2012; 10: 44.
 26. Ricci G, Granzotto M, Luppi S, et al. Effect of Seminal Leukocytes on In Vitro Fertilization and Intracytoplasmic Sperm Injection Outcomes. *Fertil Steril* 2015; 104: 87–93.