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Correlation between Sperm Analysis Factors and Pregnancy in Post-Varicocele Surgery Patients with Microligation Technique

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ABSTRACT

Background: Varicocele is the most common cause of male infertility, with a prevalence of 19-41% in men with primary infertility and 45-81% in men with secondary infertility. However, further research is needed to investigate its impact on postoperative pregnancy outcomes. ***Methods:*** This study employed a quantitative research design with a case-control approach to evaluate sperm analysis-related factors influencing pregnancy in patients who underwent varicocelectomy using the microligation technique. Patient data were obtained from medical records at Prof. Dr. IGNG Ngoerah General Hospital and Balimed Denpasar Hospital from 2018 to 2022, with a minimum sample size of 14 individuals in each group based on inclusion and exclusion criteria. Data analysis was conducted using SPSS v21.0 to determine the relationship between sperm analysis and pregnancy occurrence in couples. ***Results:*** Significant differences were found in the duration of infertility between the case and control groups (2.76 ± 2.12 vs 1.32 ± 0.62; p=0.017). The cutoff duration of infertility was determined to be 2.5 years with an AUC of 0.678 and was significantly associated with pregnancy occurrence (OR 18.857; p=0.001; 95%CI 2.195 - 181.985). Parameters such as rapid motility, slow motility, morphology, and sperm concentration exhibited significant differences between pre and post-microligation (p<0.05). Multivariate analysis revealed that the history of infertility had a 1,411 times higher impact on sperm quality compared to a shorter duration of infertility (p=0.034). ***Conclusion:*** The microligation technique was able to improve sperm analysis parameters postoperatively. However, no significant correlation was observed between these parameters and pregnancy occurrence.

Keywords: microligation; pregnancy; sperm analysis; varicocele surgery.

INTRODUCTION

Varicocele is a cause of testicular infertility with a prevalence of 19-41% in men with primary infertility and 45-81% in men with secondary infertility.[1] Based on EAU in 2019, of 12,945 infertile respondents, 1,916 respondents (14.8%) experienced varicocele. Furthermore, of 1,446 respondents who experienced azoospermia, 158 respondents (10.9%) were caused by varicocele.[2]

Several surgical techniques for the management of varicocele have been commonly used such as open inguinal (Ivanissevich technique) or retroperitoneal high ligation (Palomo technique), laparoscopic repair, *microsurgical* inguinal or subinguinal varicocelectomy, and radiological techniques such as sclerotherapy and embolization.[3,4] Of the several surgical techniques for the management of varicocele, the microligation technique has several advantages, namely based on the recurrence rate of varicocele as a postoperative complication, varicocelectomy with microsurgery is better than non-microsurgical methods.[5]

It has been reported that varicocele recurrence is generally the result of incomplete ligation of collateral veins, and enlargement of the spermatic cord field of view with a microscope during surgery can reduce the possibility of this complication. Complete dissection of the spermatic cord using an operative microscope is known to be effective for chronic scrotal pain because the procedure can result in partial testicular denervation.[6] Other studies have found that this microligation technique can also reduce the recurrence rate <1%.[7]

Previous studies have shown that there was an increase in the average sperm concentration from 12 ± 19 million/mL to 23 ± 29 million/mL after varicocelectomy (P<0.001). There was also an increase in the average sperm motility from 26% ± 16% to 32% ± 18% (P<0.001), and the average normal morphology from 5% ± 7% to 6% ± 6% (P<0.001). After microligation surgery, 23 (55%) patients achieved pregnancy, with details of 11 (26%) experiencing spontaneous pregnancy, 1 (2%) with intrauterine insemination, and 11 (26%) with in-vitro fertilization.

Among the 20 patients with severe oligospermia (<5 million/mL) preoperatively, there was a statistically significant improvement in sperm concentration, motility, and morphology postoperatively (P<0.001).[8] Other studies have shown that patients with varicocele who underwent microligation surgery had the highest pregnancy rates of 41.97% with the lowest recurrence rate (1.05%) and hydrocele complications (0.44%) compared to the Palomo technique, laparoscopy, and open inguinal.[9] However, the microligation surgery technique still needs to be studied further regarding its impact on postoperative pregnancy. Therefore, the author would like to discuss further the factors of sperm analysis on pregnancy in post-varicocele surgery patients with the microligation technique.

METHOD

This study used a quantitative research design with a case-control design. The study was conducted from July 2022 to July 2023 at Prof. Dr. IGNG Ngoerah General Hospital. The sample was patients who had undergone varicocele surgery with the microligation technique registered at Prof. Dr. IGNG Ngoerah General Hospital from 2018 to 2022, where the patient and female partner were <35 years old. This study excluded patients with scrotal pain, female partners who were diagnosed as infertile and using contraception, and patients who underwent varicocele surgery for cosmetic reasons. The sample was categorized as a case group if the couple had not become pregnant after undergoing varicocele surgery, while the control group was a couple of patients who successfully became pregnant after the patient underwent varicocele surgery. The sample was taken using a random sampling technique with a minimum of 14 people per group. This study assessed sperm analysis parameters such as total sperm motility, progressive sperm motility, sperm morphology, and total sperm count. Other variables such as age, BMI, smoking habits, alcohol drinking habits, duration of infertility, varicocele location, duration of surgery, and internal spermatic vein as control variables.

Data were analyzed using SPSS 21.0 software, where numerical data will be presented in the form of mean ± SD (if the data is normally distributed) or median (range) (if the distribution is not normal). Nominal data will be presented in the form of percentages or proportions.

Comparison of numerical variables will be done using the unpaired T-test or its alternative, the Mann-Whitney test. Categorical variables (nominal and ordinal) will be analyzed using the X^2 test. Then, the classification of variables from sperm analysis is carried out using the mean or median value to classify the variables into binary. Furthermore, a cross-tabulation test is carried out with X^2 to calculate the Odds Ratio (OR). All variables that have a p-value <0.25 in relation to the case and control groups (failed pregnancy vs pregnant) will be further analyzed using multivariate logistic regression analysis which will obtain the Adjusted OR value and 95% confidence interval. The P-value <0.05 is considered significant.

RESULT

Characteristics of Research Samples

This study involved 50 patients who underwent microligation varicocelectomy surgery, divided into 25 patients in the case and control groups. The average age of the sample in the case group was 31.08 ± 3.71 years, while in the control group, it was 29.48 ± 2.58 years. There was a smoking habit in both groups with an average duration of smoking of 4.92 ± 6.40 years in the case group, and 6.24 ± 7.84 years in the control group. Based on the distribution of smoking history, the prevalence of smoking in the case group (44%) was higher than in the control group (40%).

Based on the location of varicocele, all samples (25 people) in the case group experienced right and left varicocele, while in the control group, there were 24 people experiencing bilateral varicocele and 1 person experiencing right varicocele. Based on the analysis of sperm quality, it was found that both groups experienced an increase in values from pre-intervention compared to post-intervention of all parameters. However, the control group was found to have mostly higher post-intervention sperm analysis parameter values than the case group. In general, there was no difference in the characteristics of the study samples between the case and control groups (p>0.05). The sperm analysis was also obtained with comparable values between the case and control groups (p>0.05). Only the duration of infertility was significantly different (p=0.017), where the case group had a higher average than the control group (2.76 ± 2.12 vs. 1.32 ± 0.62) (Table 1).

TABLE 1: Patient characteristics and sperm analysis in case and control groups.

Variables	Cases (n=25)	Control (n=25)	Mean Difference	p-value
Age patients (years), mean ± SD	31.08 ± 3.71	29.48 ± 2.58	1.60	0.084 [b]
Smoking history				
Yes	11 (44%)	10 (40%)		0.774 [a]
No	14 (56%)	15 (50%)		
Alcohol history				
Yes	9 (36%)	15 (60%)		0.089 [a]
No	16 (64%)	10 (40%)		

Variables	Cases (n=25)	Control (n=25)	Mean Difference	p-value
Varicocele location				
Right	0 (0%)	1 (2%)		1,000 [a]
Left	0 (0%)	0 (0%)		
Both	25 (100%)	24 (96)		
Patient BMI (kg/m ^{2}), mean±SD	23.15 ± 2.65	21.75 ± 2.31	1.40	0.052 [b]
Duration of smoking (years), mean±SD	4.92 ± 6.40	6.24 ± 7.84	1.32	0.707 [c]
Duration of infertility (years), mean±SD	2.76 ± 2.12	1.32 ± 0.62	1.44	0.017* [c]
Operation time (minutes), mean±SD	30.40 ± 6.91	29.60 ± 8.02	0.80	0.447 [c]
VSI left, mean±SD	6.84 ± 1.86	7.04 ± 1.90	0.20	0.890 [c]
VSI right, mean±SD	7.56 ± 2.39	7.04 ± 2.13	0.52	0.271 [c]
Pre-op sperm analysis				
Fast motility (%), mean±SD	4.52 ± 6.33	3.64 ± 11.76	0.88	0.129 [c]
Slow motility (%), mean±SD	27.00 ± 13.05	26.32 ± 14.51	0.68	0.862 [b]
Morphology (%), mean±SD	5.28 ±11.05	3.20 ± 3.77	2.08	0.968 [c]
Sperm concentration (million), mean±SD	26.66 ± 32.22	20.70 ± 20.37	5.96	0.977 [c]
Post-op sperm analysis				
Fast motility (%), mean±SD	9.28 ± 14.29	5.84 ± 12.10	3.44	0.402 [c]
Slow motility (%), mean±SD	33.52 ± 15.96	37.48 ± 11.93	3.96	0.326 [b]
Morphology (%), mean±SD	7.36 ± 17.13	12.00 ± 22.53	4.64	0.289 [c]
Sperm concentration (million), mean±SD	28.57 ± 18.80	32.53 ± 18.48	3.95	0.600 [c]

Note: *statistically significant (p<0.05); [a] analysis with *Pearson Chi-Square test;* [b] analysis with *independent T-test;* [c] analysis with *Mann-Whitney test.*

ROC Analysis of Sperm Analysis Results after Surgery

All sperm analysis parameters have varying cut-off values, but these values have insignificant p-values (p>0.05) (Table 2 and Figure 1). In the variable of infertility duration, there are opposing results, where the cut-off value of 2.5 years has a significant value with a sensitivity of 44% and a specificity of 96%.

The results of the ROC analysis of post-op sperm are not significant, but based on the p-value occupying the two lowest and the narrow 95%CI range of all sperm parameters, morphology, and fast motility have the potential to be sperm analysis parameters that need to be considered after varicocele surgery.

TABLE 2: ROC results of sperm analysis after surgery.

Variables	Cut-Off	AUC	Sn	Sp	p-value	95%CI	
						Lower	Upper
Rapid motility	2.5	0.568	64%	48%	0.410	0.407	0.729
Slow motility	35.5	0.437	52%	44%	0.443	0.274	0.600
Morphology	3.5	0.414	56%	32%	0.295	0.254	0.573
Sperm concentration	22.27	0.457	60%	44%	0.600	0.295	0.619
Infertile period	2.5	0.678	44%	96%	0.031	0.526	0.829

Description: AUC: area under the curve; Sn: sensitivity; Sp: specificity; CI: confidence interval.

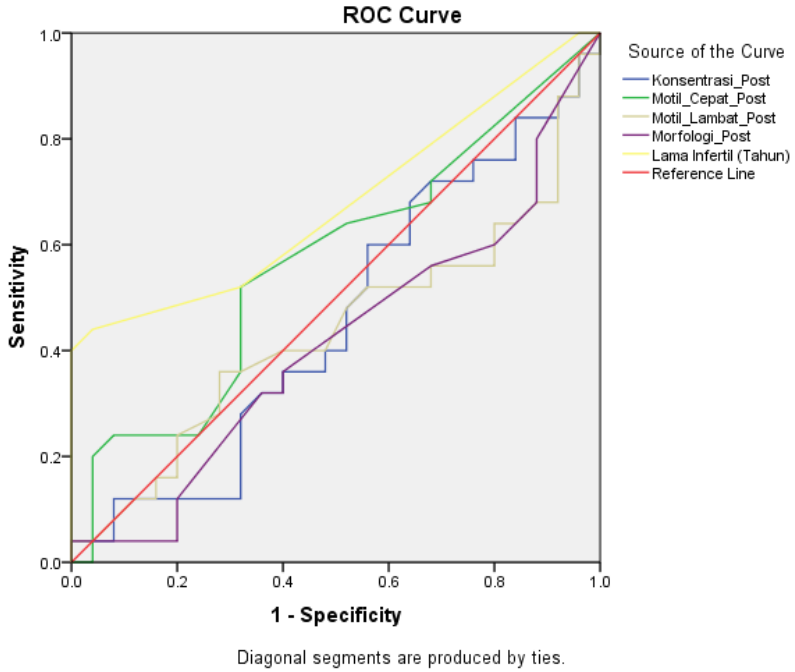

FIGURE 1: ROC curve of sperm analysis results after surgery.

Bivariate Analysis based on ROC Curve *Cut-off*

Infertile duration with a cut-off of 2.5 years was significantly associated with pregnancy (OR 18.857; p=0.001). This means that infertile duration <2.5 years has an 18 times greater chance of pregnancy after varicocelectomy. Sperm analysis parameters such as fast motility, slow motility, concentration, and sperm morphology were found to be unrelated to pregnancy. The results of the bivariate analysis of the ROC curve cut-off, post-op sperm parameters were not associated with pregnancy, but based on the p-value obtained, morphology and fast motility have the potential to be sperm analysis parameters that affect pregnancy (Table 3).

TABLE 3: Bivariate analysis based on ROC curve cut-off.

Variables	Case (Not Pregnant)	Control (Pregnant)	OR	p-value	95%CI
Rapid motility					
<2.5%	9 (36)	12 (48)	0.609	0.390	0.196 – 1.891
>=2.5%	16 (64)	13 (52)			
Slow motility					
<35.5%	12 (48)	11 (44)	1,175	0.777	0.386 – 3.576
>=35.5%	13 (52)	14 (56)			
Morphology					
<3.5%	11 (44)	8 (32)	1,670	0.382	0.527 – 5.290
>=3.5%	14 (56)	17 (68)			
Sperm concentration					
<22.27 million	10 (40)	11 (44)	0.848	0.774	0.276 – 2.611
>= 22.27 million	15 (60)	14 (56)			
Infertile Period					
>=2.5 years	11 (44)	1 (4)	18,857	0.001	2,195 – 181,985
< 2.5 years	14 (56)	4 (96)			

Description: OR: odd ratio; CI: confidence interval.

Differences in Sperm Analysis Results Before and After Surgery

In general, the values of fast and slow sperm motility, sperm morphology, and post-op sperm concentration were significantly higher. In addition, there was a significant difference between pre-op sperm analysis, which included fast motility (p=0.036), slow motility (p=0.000), morphology (p=0.001), and sperm concentration (p=0.002) and the results of post-op sperm analysis (Table 4). This shows that treatment with microligation varicocelectomy can improve and increase the value of post-operative sperm analysis results.

TABLE 4: Differences in sperm analysis results before and after surgery (Pre-op vs Post-op).

Variables	Pre-Op (n=25)	Post-Op (n=25)	p-value
Fast motility (%), mean±SD	4.08±9.36	7.56±13.22	0.036 [b]
Slow motility (%), mean±SD	26.66±13.66	35.50±14.09	0.000 [a]
Morphology (%), mean±SD	4.24±8.24	9.68±19.94	0.001 [b]
Sperm concentration (million), mean±SD	23.68±26.86	30.55±18.55	0.002 [b]

Note: *statistically significant (p<0.05); [a] analysis with Paired T-test; [b] analysis with Wilcoxon Test.

Correlation of Postoperative Sperm Parameters to Pregnancy Time

There was no significant correlation between fast motility (p=0.175), slow motility (p=0.213), morphology (p=0.290), sperm concentration (p=0.179), and pregnancy time (Table 5).

Based on the correlation analysis, morphological parameters can be considered as parameters that need to be considered in post-varicocele surgery patients because they are linearly related to pregnancy in theory.

TABLE 5: Correlation analysis of post-operative sperm parameters using the *micro-ligation method* against pregnancy time.

Post-Op Sperm Parameters (N=25)	*Microligation* Method	
	r	P
Rapid motility	-0.280	0.175 [a]
Slow motility	-0.258	0.213 [a]
Morphology	0.220	0.290 [a]
Sperm concentration	-0.278	0.179 [a]

[a] Spearman correlation test; *p-value is said to be significant if ≤0.05.

Multivariate Analysis

The duration of infertility is a variable that can independently affect sperm quality, the relationship between the two was also found to be significant (p=0.034). It was found that the adjusted odds ratio (OR) for the duration of infertility was 1.411 with a 95%CI value of 1.181 - 1.936. This means that patients with a long history of infertility affect sperm quality 1.411 times higher than those with a short history of infertility. Other confounding variables were not significantly related to the incidence of pregnancy (p> 0.05) (Table 6).

TABLE 6: Results of multivariate analysis.

	B	SE	95%CI	Adjusted OR	p-value
Age	0.127	0.156	0.836 – 1.541	1,135	0.415
Alcohol History	-0.437	0.753	0.148 – 2.825	0.646	0.562
Body Mass Index	-0.075	0.149	0.693 – 1.243	0.928	0.617
Infertile period	-0.888	0.419	1,181 – 1,936	1,411	0.034*
Rapid Motility	-0.014	0.032	0.926 – 1.050	0.986	0.660

Note: *statistically significant (p<0.05).

DISCUSSION

This study evaluated two groups of patients who had undergone varicocele correction using the microligation method and aimed to identify factors in sperm analysis, especially sperm concentration, motility, and sperm morphology that affect pregnancy success. In this study, it was found that the three variables did not have significant differences between the two groups of patients.

Several studies have been reported in Indonesia and Bali. Duarsa, et al evaluated the factors that contribute to spontaneous pregnancy after varicocelectomy showing that progressive sperm motility before varicocelectomy (≥37.5%) is a factor associated with spontaneous pregnancy after varicocelectomy.[10,11]

Then, Birowo et al., reported that varicocele correction causes an increase in sperm concentration, sperm motility, and improvement in sperm morphology. This study also evaluated the level of sperm DNA fragmentation which is a new indicator to predict sperm functionality and pregnancy rates. [12]DNA fragmentation in sperm from varicocele patients has been associated with high rates of pregnancy failure and/or live birth percentage.[6,13] Other factors that improve after varicocelectomy are decreased testicular vein hydrostatic pressure, reflux of toxic metabolites, and testicular temperature which can then improve Leydig and Sertoli cell function which has an impact on sperm motility.[14,15]

In relation to pregnancy success, varicocele correction has been consistently proven to be related to pregnancy success. However, the parameters of sperm analysis that affect pregnancy tend to vary in several studies. In this study, there has been no significant relationship between sperm analysis results and pregnancy events. However, the p-value in bivariate analysis shows that morphology and rapid motility have the potential to affect pregnancy events.

Clinically, the presence of normal sperm motility and morphology provides evidence of fertility potential in infertile patients. Based on the results of a study conducted on 4,500 normozoospermic men from 14 different countries, the baseline values of normal sperm characteristics have been established by WHO.[16] Similar to oligozoospermia, asthenozoospermia is highly correlated with infertility, indicating that motility is an equally important semen parameter for achieving pregnancy. A previous study found that the natural conception subgroup (n = 5126) had a conception rate of 59.5%, and the median time to conception was 27 (26–30) months. The chance of conception was 65% greater for men with progressive motility ≥32% (HR: 1.65, 95%CI: 1.47–1.84) and a value of 40% for progressive motility could be used to predict the chance of conception over 5 years.[17,18]

Data on the impact of sperm morphology on the outcome of natural conception are limited. One study conducted a retrospective medical record review investigating the likelihood of achieving pregnancy without ART in men with severe teratozoospermia, i.e. 0% normal forms, according to strict Kruger criteria. Twenty-four men with 0% normal forms were compared with 27 randomly selected men with ≥4% normal forms over a 3-year period. Although the natural conception rate was higher in men with ≥4% normal forms compared with the severe teratozoospermia group (51.8% vs. 25%, $p \leq 0.05$), men with 0% normal forms were still able to conceive naturally in 25% of cases. Furthermore, in cases where men with 0% normal forms did conceive naturally, 100% of these men had another child via natural conception. The researchers concluded that strict morphology should not be used to predict the potential for fertilization, pregnancy, or live birth, and in men with 0% normal shape, alternative reproductive modalities should be considered before direct IVF is undertaken.[19]

Duarsa, et al., reported that progressive sperm motility before varicocelectomy was the most influential factor[11], while Fallara et al., stated that sperm concentration was a factor associated with spontaneous pregnancy.[20] However, Mahdi et al., reported that several aspects of sperm parameters such as sperm concentration, sperm motility, percent motile sperm, and percent sperm with normal morphology had significant effects.[21] The latest pregnancy rate indicator in varicocele patients removed in the medical world is the level of DNA fragmentation in sperm which is currently still in the process of validation and diagnostic research.[13]

Despite the results obtained, there are several shortcomings in this study that need to be considered. First, this study was concentrated in one area (Denpasar) where the demographic characteristics of different areas (rural areas) can affect the success rate of pregnancy after varicocele correction differently. Second, ethnicity was also not controlled in this study so the effect of genetic diversity in different races cannot be excluded. Then, serial semen examination is needed to monitor patient development over time and allow for intra-group analysis (follow-up study).

CONCLUSION

There were significant differences in motility, morphology, and total sperm count between the condition of varicocele patients before microligation surgery compared to the condition of varicocele patients after microligation surgery. However, these parameters were not significantly related to the incidence of pregnancy in couples after surgery with the microligation method.

ETHICAL CONSIDERATIONS

This research has obtained approval from the Research Ethics Commission Unit, Faculty of Medicine, Udayana University with letter number 1663/UN14.2.2.VII.14/LT/2023.

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