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ORIGINAL ARTICLE

Male Fertility

# Effect of varicocele repair on sperm retrieval rate and testicular histopathological patterns in men with nonobstructive azoospermia

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Varicocele adversely affects semen parameters. However, the effect of varicocele repair on the sperm retrieval rate and testicular histopathological patterns in men with nonobstructive azoospermia has not been widely reported. We retrospectively assessed the sperm retrieval rates and testicular histopathological patterns in men with nonobstructive azoospermia who were referred to the Urology Clinic in Dr. Cipto Mangunkusumo Hospital (Jakarta, Indonesia) and Bunda General Hospital (Jakarta, Indonesia) between January 2009 and December 2019. We compared patients who had undergone a surgical sperm retrieval procedure for assisted reproductive technology no earlier than three months after varicocele repair and those who had not undergone varicocele repair. The study included 104 patients (age range: 26–54 years), 42 of whom had undergone varicocele repair before the sperm retrieval procedure and 62 who had not. Motile spermatozoa were found in 29 (69.1%) and 17 (27.4%) patients who had undergone varicocele repair before the sperm retrieval procedure and those who had not undergone the repair, respectively (relative risk: 2.51; 95% confidence interval: 1.60–3.96;  $P < 0.001$ ). A predicted probabilities graph showed consistently higher sperm retrieval rates for patients with varicocele repair, regardless of their follicle-stimulating hormone levels. Patients who underwent varicocele repair showed higher testicular histopathological patterns ( $P = 0.001$ ). In conclusion, men with nonobstructive azoospermia and clinical varicocele who underwent varicocele repair before the sperm retrieval procedure had higher sperm retrieval rates compared to those who did not undergo varicocele repair.

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**Keywords:** azoospermia; follicle-stimulating hormone; sperm retrieval rate; testicular histopathology; varicocele

## INTRODUCTION

Varicocele refers to the dilation or tortuosity of the pampiniform plexus of the spermatic veins.<sup>1</sup> It is a common finding in infertile couples, affecting 15%–40% of men with primary infertility<sup>2,3</sup> and 5%–10% of men with azoospermia.<sup>4</sup>

Varicocele results in decreased testosterone secretion, impaired spermatogenesis, and testicular atrophy.<sup>5</sup> As it is mostly asymptomatic, men with varicocele usually seek medical help due to abnormal semen parameters.<sup>6</sup> While several studies have demonstrated improvement of sperm parameters in ejaculated semen samples after varicocele repair,<sup>7,8</sup> the effect of varicocele repair in improving spermatogenesis among men with nonobstructive azoospermia (NOA) is still not well established, especially among Asian men, who tend to have higher follicle-stimulating hormone (FSH) levels and lower testis size.<sup>9–11</sup> Assisted reproductive technology (ART) by *in vitro* fertilization or intracytoplasmic sperm injection is often needed for fertilization in couples with this condition. In most cases, a surgical sperm retrieval procedure using testicular sperm extraction (TESE) is needed to retrieve spermatozoa in these patients. Furthermore, testicular histopathological findings should be considered in men with NOA seeking fertility through sperm retrieval procedures.<sup>12,13</sup> This study aimed to investigate the sperm retrieval rate (SRR) and testicular

histopathological patterns among men with NOA after varicocele repair.

## PATIENTS AND METHODS

### Patients

This retrospective study included men with NOA seeking medical treatment for infertility at the Urology Clinic in Dr. Cipto Mangunkusumo Hospital (Jakarta, Indonesia) and Bunda Hospital (Jakarta, Indonesia) from January 2009 to December 2019. A detailed review of the medical records for infertility evaluation was conducted for each patient. The diagnosis of NOA was based on patient history, clinical and ultrasound examination (testicular volume  $<15$  ml), and reproductive hormone (FSH  $>9.2$  mIU  $\text{ml}^{-1}$ )<sup>9</sup> assessment, and was confirmed in testicular histopathology specimens obtained during the sperm retrieval procedures. The presence of clinical varicocele was diagnosed by physical examination and, in some cases, was confirmed by scrotal ultrasonography. Patients with subclinical varicocele were excluded from the study. Physical examination was performed in an upright position by the same examiner. Patients were asked to perform the Valsalva maneuver when the varicocele was not clearly palpable. Azoospermia is defined as the absence of spermatozoa in the seminal fluid after centrifugation in two consecutive semen analyses

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after 2–7 days of sexual abstinence. FSH levels were measured using an immunochemiluminescence assay (Roche Diagnostics GmbH, Mannheim, Germany) as part of the reproductive hormone assessment. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (approval number: KET-812/UN2.F1/ETIK/PPM.00.02/2020) and complies with the Code of Ethics of the World Medical Association (Declaration of Helsinki 1964, revised 2003 and Declaration of Tokyo 1975, revised 20064). No consent was obtained from the participants due to its retrospective study.

There were two groups of patients: those who underwent the sperm retrieval procedure without prior varicocele repair (Group 1) and those who had undergone varicocele repair no earlier than three months before the sperm retrieval procedure (Group 2). All patients were undergoing ART programs at our centers and the decision of each patient's treatment, *i.e.*, whether the patient underwent varicocele repair before sperm retrieval or sperm retrieval procedure without varicocele repair, was based on the patient's preference after discussion with the urologist.

**Varicocele repair**

Varicocele repair was performed using a microsurgical subinguinal approach by the same operator. A standard operating microscope (Carl Zeiss Meditec AG, Jena, Germany) with a dual ocular system was used in all procedures. Incisions were made at the level of the external inguinal ring until the subcutaneous tissue was reached. A pair of Senn-Mueller retractors (B. Braun, Melsungen, Germany) followed by a pair of Parker-Langenbeck retractors (B. Braun) were used to help with visualization of the surgical field. After identification, the spermatic cord was elevated using a Babcock clamp (B. Braun) and was secured above the skin level with a Senn-Mueller retractor placed under and perpendicular to the spermatic cord. Enlarged veins were identified and ligated. This technique was used in all patients regardless of varicocele grades.

**Sperm retrieval procedure**

All sperm retrieval procedures were conducted using TESE. TESE was performed by making a small incision on the testis, followed by the collection of a small portion of the testicular tissue. The collected tissue was then placed in G-MOPS™ PLUS medium (Vitrolife, Göteborg, Sweden) containing human serum albumin and was examined using a standard laboratory microscope. The SRR was defined as the number of successful sperm retrievals, *i.e.*, retrievals showing the presence of viable spermatozoa, in relation to the total number of sperm retrievals.

**Testicular histopathology**

Testicular biopsies were performed during the sperm retrieval procedure using a standard procedure for each testicle, and sampling from the same location used for TESE. The specimens were analyzed by the same laboratory analyst. Testicular histopathology was reported in terms of five patterns based on the Johnsen scoring system (Figure 1): histology representing normal spermatogenesis, hypospermatogenesis (reduction in the number of normal spermatogenic cells), late maturation arrest (arrest at the spermatid stage), early maturation arrest (arrest at the spermatocyte stage), and Sertoli cell-only (SCO; indicating an absence of germ cells).<sup>14,15</sup>

**Statistical analyses**

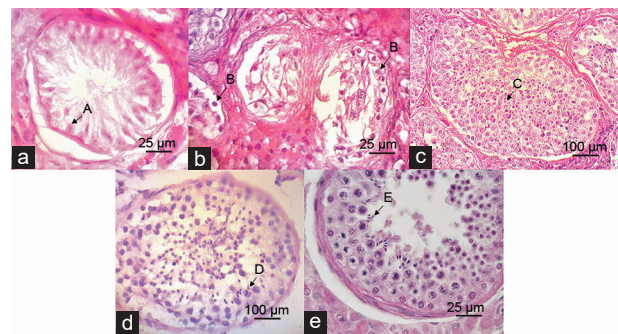
The differences in SRR values between Group 1 (sperm retrieval only) and Group 2 (varicocele repair + sperm retrieval) are presented as relative risks. Comparisons were analyzed using Student's

*t*-test or the Mann–Whitney U test for continuous data, and the Chi-square test for categorical data. A predicted probability of the main outcomes was constructed based on logistic regression analysis. All statistical analyses were performed with SPSS version 20.0 (IBM Corp., Armonk, NY, USA); *P* < 0.05 was considered statistically significant.

**RESULTS**

The study included 104 patients, 62 (59.6%) of whom underwent the sperm retrieval procedure without prior varicocele repair (Group 1), while 42 (40.4%) patients underwent varicocele repair before the sperm retrieval procedure (Group 2). The range of patient age was 26–54 years, and there was no significant age difference between patients in Groups 1 and 2. Overall, motile spermatozoa were successfully retrieved from 46 patients during the sperm retrieval procedures, yielding a total SRR of 44.2%. The patient characteristics and sperm retrieval outcomes are shown in Table 1.

There was a higher number of successful motile spermatozoa retrievals in Group 2 compared to Group 1. Our results demonstrated that patients who underwent varicocele repair before the sperm retrieval procedure showed higher SRRs compared to those who underwent sperm retrieval only (relative risk [RR]: 2.51; 95% confidence interval [CI]: 1.60–3.96; *P* < 0.001). Furthermore, we performed an interaction analysis on the SRRs for the two groups based on FSH levels, which showed a heterogeneous result (*P*-value for Mantel-Haenzel statistics = 0.001) and prompted the construction of a predicted probabilities graph based on the mean FSH level for each group (Figure 2). The analysis showed a consistent SRR trend in relation to the FSH levels between the two groups, *i.e.*, patients



**Figure 1:** Five testicular histopathological patterns based on spermatogenesis. (a) Sertoli cell-only (A). (b) Early maturation arrest with spermatocyte cells (B). (c) Late maturation arrest with spermatids (C). (d) Hypospermatogenesis, which is marked by decreased number of normal spermatozoa (D). (e) Normal spermatogenesis (E).

**Table 1: Comparison of patients' characteristics and sperm retrieval rates in the study groups**

Characteristic	Group 1 (n=62)	Group 2 (n=42)	P
Age (year), mean±s.d.	36.9±6.1	37.3±5.3	NS <sup>a</sup>
Testicular volume (ml), mean±s.d.	6.89±1.03	7.12±0.62	NS <sup>a</sup>
FSH level (mIU ml <sup>-1</sup> )			
Mean±s.d.	16.4±9.7	12.8±8.8	NS <sup>a</sup>
Median (IQR)	17.14 (7.69-22.48)	11.34 (6.92-15.70)	NS <sup>a</sup>
Motile spermatozoa, n (%)	17 (27.4)	29 (69.1)	<0.001 <sup>c</sup>

<sup>a</sup>*P*-value for Student's *t*-test; <sup>b</sup>*P*-value for Mann-Whitney U test; <sup>c</sup>*P*-value for Chi-square test. Group 1: no varicocele repair before sperm retrieval procedure; Group 2: varicocele repair before sperm retrieval procedure. FSH: follicle-stimulating hormone; IQR: interquartile range; s.d.: standard deviation; NS: not significant

who had varicocele repair before the sperm retrieval procedures had higher SRRs compared to those who did not undergo varicocele repair.

Testicular histopathological analyses in the two study groups found that most patients showed late maturation arrest patterns. The SCO pattern was only observed in Group 1. None of the patients presented the hypospermatogenesis pattern (Table 2). Furthermore, an analysis of SRR in relation to histopathology showed that patients who showed the late maturation arrest pattern had the highest SRR, while patients with SCO had the lowest SRR (Table 3).

**DISCUSSION**

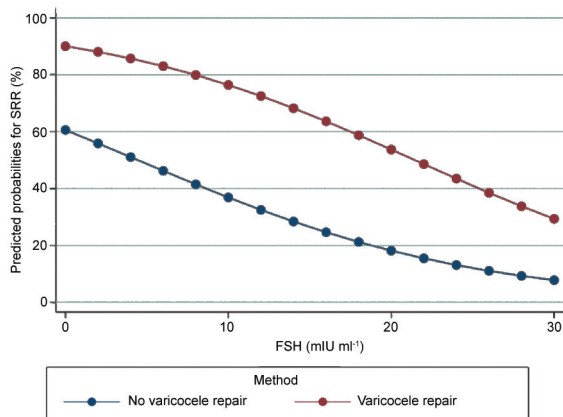
Our results showed that patients who underwent varicocele repair before a sperm retrieval procedure had higher SRRs compared to those who did not, irrespective of their FSH levels. Furthermore, the analysis of testicular histopathological patterns revealed that most patients who underwent varicocele repair showed better spermatogenic cell numbers and composition based on the Johnsen scoring system compared to those who did not. These findings should assist clinicians in determining the treatment for couples who have male-factor infertility, particularly patients with NOA, who seek fertilization by ART. Apart from the clinical benefit, *i.e.*, a higher chance of successful

sperm retrieval, varicocele repair also has an economic benefit, as patients might be able to undergo fewer sperm retrieval procedures if they have had prior varicocele repair, especially as neither procedure is covered by most insurance providers in Indonesia.

When encountering patients with azoospermia, a detailed assessment is needed to correctly diagnose the underlying cause and instigate a correct treatment plan. When semen analysis showing azoospermia is not performed by a specialized fertility laboratory, it is advisable to repeat the semen analysis in a laboratory that regularly performs analyses using the World Health Organization Laboratory Manual for the Examination and Processing of Human Semen (5<sup>th</sup> edition).<sup>16</sup> Patients are advised to maintain sexual abstinence for 2-7 days before providing samples for semen analysis.<sup>17</sup> In this study, two consecutive azoospermia results from semen analysis were needed for inclusion. Furthermore, there were two additional defining criteria when initially diagnosing NOA: the FSH level and the testis size. As described in previous studies, most notably by Schoor *et al.*,<sup>18</sup> the combination of high FSH level (>7.6 IU l<sup>-1</sup>) and small testis size (long axis ≤4.6 cm) accurately predicts spermatogenesis dysfunction as the cause of infertility. However, these cut-off values for FSH level and testis size were based on the Caucasian population. Asian men tend to have higher FSH levels and smaller testes; therefore, it is essential to use reference values specific to the Asian population.<sup>9-11</sup> Huang *et al.*<sup>9</sup> investigated the combination cut-off values for FSH level and testis size to predict the cause of azoospermia in an Asian population. Based on their study, the positive predictive value of a combination of FSH >9.2 mIU ml<sup>-1</sup> and testicular volume <15 ml for NOA was 99.2%. Thus, FSH >9.2 mIU ml<sup>-1</sup> and testicular volume <15 ml were used as the inclusion criteria for NOA in our study, before confirmation by the testicular histopathological results. A minimum three-month period between the varicocele repair and the sperm retrieval procedure was chosen in this study because it represents the minimum period allowing the improvement of sperm parameters after varicocele repair to be observed. This is based on the time for completion of the spermatogenesis process, which occurs within a range of 64 days to 74 days.<sup>11,14</sup> Thus, in several centers, the recommended interval between varicocele repair and the sperm retrieval procedure is 3–23 months.<sup>19</sup>

The detrimental effect of varicocele on male fertility has been widely studied, showing that pathogenesis principally results from the induction of spermatogenesis impairment due to obstructed drainage or blood pooling that causes high scrotal temperature. An increase in scrotal temperature leads to DNA and protein damage, particularly in the spermatid nucleus, Leydig cells, and tubule cells.<sup>5,10</sup>

Our study found that men with NOA who underwent varicocele repair before the surgical sperm retrieval procedure had higher SRRs compared to those who underwent the surgical sperm retrieval procedure only. This result is comparable with those of previous studies, which investigated the outcome of varicocele repair in men with NOA,<sup>19-22</sup> in which the SRRs among men with azoospermia after varicocele repair ranged between 30% and 54.5%.<sup>13,23,24</sup> A meta-analysis by Esteves *et al.*<sup>19</sup> comprising 468 patients reported that patients with NOA who underwent varicocele repair before the sperm retrieval procedure had higher SRRs compared to those who did not (odds ratio [OR]: 2.65; 95% CI: 1.69–4.14). Furthermore, the SRR has been reported to be unrelated to the degree of varicocele.<sup>20</sup> Other studies which reported sperm parameter improvement following varicocele repair include those by Weedin *et al.*,<sup>25</sup> who reported a 32% semen parameter improvement after varicocele repair, and Abdel-Meguid *et al.*,<sup>26</sup> who observed that varicocele repair improved



**Figure 2:** Predicted probabilities for SRR based on FSH level between patients who did not undergo varicocele repair and patients who had varicocele repair. *P* < 0.001. SRR: sperm retrieval rate; FSH: follicle-stimulating hormone.

**Table 2: Comparison of testicular histopathology patterns in the study groups**

Histopathological pattern	Group 1 (n=62), n (%)	Group 2 (n=42), n (%)	<i>P</i>
Late maturation arrest	27 (43.6)	27 (64.3)	0.001
Early maturation arrest	20 (32.3)	15 (35.7)	
Sertoli cell-only	15 (24.2)	0 (0)	

*P*-value for Fisher's exact test. Group 1: no varicocele repair before sperm retrieval procedure; Group 2: varicocele repair before sperm retrieval procedure

**Table 3: Sperm retrieval rates based on testicular histopathological patterns (n=104)**

Histopathological pattern	Sperm retrieval, n (%)		<i>P</i>
	Successful	Unsuccessful	
Late maturation arrest	37 (68.5)	17 (31.5)	<0.001
Early maturation arrest	7 (20.0)	28 (80.0)	
Sertoli cell-only	2 (13.3)	13 (86.7)	

*P*-value for Chi-square test



the semen concentration by 15%, motility by 15.2%, and morphology by 8.0%, although this was not exclusive to men with azoospermia.

We also demonstrated that the difference in the SRR between the two groups showed a consistent trend in relation to increasing FSH levels. In men with azoospermia, the FSH level is a helpful tool in differentiating between obstruction and spermatogenesis impairment as the etiology of azoospermia. There have been conflicting results from past studies as to whether the FSH level is associated with sperm retrieval success in NOA. Bromage *et al.*<sup>27</sup> reported that an elevated FSH level (FSH >10 IU l<sup>-1</sup>) along with small testicular size (long axis <4 cm) is associated with lower SRR compared to patients with normal FSH (FSH <10 IU l<sup>-1</sup>;  $P = 0.0001$ ). Similarly, Souza *et al.*<sup>28</sup> found that FSH levels can serve as a predictor for motile sperm recovery using biopsy in NOA patients, in which FSH levels <16.05 IU l<sup>-1</sup> were significantly associated with the finding of motile spermatozoa ( $P < 0.05$ ). In contrast, a study by Ramasamy *et al.*<sup>29</sup> on 792 men with NOA found that higher SRRs were observed in NOA patients with FSH >15 IU ml<sup>-1</sup> compared to those with FSH <15 IU ml<sup>-1</sup> ( $P = 0.006$ ). Furthermore, a study on 395 men with NOA by Tournaye *et al.*<sup>30</sup> reported that the serum FSH level is not a strong predictive factor in determining the success of sperm recovery procedures. Despite these reports, our study demonstrated that higher FSH levels correlated with lower SRRs in both study groups, with a consistent trend.

Few studies have investigated how the histopathological profile affects the SRR. It has been previously reported that men with high FSH levels and small testes are more likely to present a heterogeneous pattern of seminiferous tubules.<sup>31,32</sup> A study comprising 17 patients reported a 100% success rate in patients with hypospermatogenesis and maturation arrest, but only a 16.6% success rate in patients with SCO.<sup>33</sup> Moreover, a study by Ustuner *et al.*<sup>4</sup> reported a significant histopathological improvement after varicocele repair in infertile men, where the average Johnsen score was significantly increased after varicolectomy. Other studies have shown improvements in spermatogenesis following varicocele repair in men with NOA based on postoperative ejaculate parameters, where spermatozoa were found mainly among patients with hypospermatogenesis.<sup>34,35</sup> The results in our study support these findings, despite the findings not being the result of a cohort study. This was a limitation of our study since the testicular histopathological patterns of patients before varicocele repair were not known. Because of this, further studies with a cohort design comparing spermatogenesis parameters before and after varicocele repair are needed to ensure consistency among various populations.

This study also has other limitations. Patients were not randomized into the study groups as the decision on whether a patient should undergo varicocele repair before the sperm retrieval procedure or not was based on the patient's preference after discussion with the urologist. Several factors that contributed to their decisions included their female partner's age and fertility status, financial considerations, and family influence. We also did not investigate the impact of varicocele grade and laterality, nor the duration of infertility, in relation to the results of this study, although these factors could provide valuable information towards the outcome and, eventually, on decision-making between clinician and patient. Furthermore, semen analysis results after varicocele repair were not reported in the study despite there being several reports on the finding of viable spermatozoa in the ejaculate following varicocele repair, including that in men with NOA.<sup>19,22,33</sup> Most of the patients in Group 2 had postoperative semen analyses performed as part of their couple-infertility program, the results of which were azoospermia. As this was not the main parameter in this study, the time interval for semen analysis after surgery varied between

these patients and was therefore not reported. We are aware that this is one of our limitations as well.

We hope that the results of this study will assist clinicians in determining the appropriate treatment for men with NOA seeking fertility, particularly among Asian populations. The findings of this study also open opportunities for further research on the association between varicocele repair and sperm retrieval, especially in NOA cases. Furthermore, a cohort study investigating the changes in spermatogenesis parameters, *i.e.*, FSH level, ejaculate sperm analysis, and testicular histopathological patterns before and after varicocele repair, will be useful to determine whether the procedure improves spermatogenesis. A prospective study should also follow the proportion of patients who achieved spontaneous pregnancy and live births after sperm retrieval, as has been recently reported.<sup>13,19,36</sup>

## CONCLUSIONS

Higher SRRs were observed among men with NOA and clinical varicocele who underwent varicocele repair prior to a sperm retrieval procedure compared to those who underwent the sperm retrieval procedure without varicocele repair.

## AUTHOR CONTRIBUTIONS

PB, DTP, WA, and NR designed the study. DTP collected the data and conducted the analysis. PB and DTP wrote the manuscript, while WA, IRS and NR reviewed and revised the discussions. DAP performed the testicular histopathological analysis. PB finalized the manuscript.

## COMPETING INTERESTS

All authors declare no competing interests.

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