Effect of microsurgical varicocelectomy on sperm DNA fragmentation in patients having infertility

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ABSTRACT
Background:
Varicocele, a prevalent cause of male infertility, is associated with impaired semen parameters and increased sperm DNA fragmentation (DFI) due to oxidative stress. Microsurgical varicocelectomy aims to improve fertility outcomes by addressing these issues.
Objective:
To evaluate the impact of microsurgical varicocelectomy on semen quality and sperm DNA integrity in men with varicocele-associated infertility.
Material and Methods:
This prospective study was conducted at Department of Urology University college of medicine and dentistry Lahore, from January 2025 to January 2026. A total of 27 infertile men with clinically palpable varicocele were included. Semen analysis was performed pre-operatively and six months post-operatively to measure sperm count, motility, morphology, and DFI. Stratified analyses were performed based on age, obesity status, duration of infertility, varicocele grade, and smoking status. Statistical analysis included paired t-tests and subgroup comparisons.
Results:
Significant improvements were observed in semen parameters post-surgery: sperm count increased from 24.46 ± 12.04 to 38.01 ± 13.88 million/mL ($p<0.001$), motility improved from 45.40 ± 11.29% to 51.15 ± 10.46% ($p=0.040$), and morphology increased from 4.90 ± 1.74% to 6.40 ± 1.73% ($p=0.002$). DFI decreased substantially from 19.37 ± 3.07% to 9.46 ± 3.22% ($p<0.001$). Stratified analysis highlighted enhanced benefits for younger, non-obese patients and those with shorter infertility durations.
Conclusion:
Microsurgical varicocelectomy significantly improves semen quality and reduces sperm DNA fragmentation, particularly in specific patient subgroups. This procedure is a vital intervention for managing varicocele-associated infertility...
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INTRODUCTION

Varicocele, a pathological enlargement of the pampiniform venous plexus in the scrotum, is a prevalent condition affecting approximately 15% of men in the general population and up to 40% of men evaluated for infertility. This condition is strongly linked to oxidative stress and increased levels of sperm DNA fragmentation (SDF), which negatively impact sperm quality and fertility potential [1,2]. Elevated SDF has been associated with reduced fertilization

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rates, poor embryo development, and recurrent pregnancy loss, making it a significant concern in reproductive medicine [3].

Microsurgical varicocelectomy, recognized as a gold standard surgical treatment for varicocele, involves precise identification and ligation of dilated veins while preserving vital structures such as the testicular artery and lymphatics. This approach significantly reduces postoperative complications and improves semen parameters, including sperm count, motility, and morphology [4,5]. The benefits of this intervention extend to a notable reduction in SDF levels, which is pivotal in enhancing fertility outcomes for affected men [6].

SDF has emerged as an important biomarker for assessing male fertility, offering insight into the extent of sperm DNA damage caused by oxidative stress, apoptosis, and environmental factors. Men with varicocele exhibit elevated levels of SDF due to increased testicular temperature, hypoxia, and reactive oxygen species (ROS) production. Microsurgical varicocelectomy effectively mitigates these factors, leading to significant improvements in sperm DNA integrity [7,8].

Clinical studies have demonstrated the efficacy of microsurgical varicocelectomy in reducing SDF levels, particularly in men with high-grade varicoceles and abnormal semen parameters. This surgical intervention has also been associated with improved outcomes in assisted reproductive technologies (ART), such as higher fertilization and live birth rates [9]. Furthermore, the procedure's impact is observed not only in improving natural conception rates but also in reducing the need for invasive ART procedures [10].

While the benefits of microsurgical varicocelectomy are well-documented, patient selection plays a crucial role in maximizing outcomes. Factors such as varicocele grade, preoperative SDF levels, and patient age are critical in determining the success of the surgery. Moreover, integrating SDF testing into routine infertility evaluations provides a more comprehensive approach to diagnosis and management, enabling personalized treatment strategies [11,12].

Varicocele is a common cause of male infertility, linked to impaired spermatogenesis, reduced semen quality, and increased sperm DNA fragmentation due to oxidative stress. While microsurgical varicocelectomy is widely recognized as an effective treatment, its specific impact on improving sperm DNA integrity and conventional semen parameters across different patient subgroups remains a critical area of exploration. This study aims to provide robust evidence on the procedure's efficacy, guiding clinical decision-making and optimizing fertility outcomes for men with varicocele-associated infertility

Material and Methods

Study Design and Setting: This prospective study was conducted at Department of Urology University college of medicine and dentistry Lahore, from January 2025 to January 2026. The study design was approved by the institutional ethical committee, and written informed consent was obtained from all participants.

Participants: The study included 27 male patients diagnosed with infertility and clinical varicocele. Inclusion criteria were patients aged 25–45 years with a history of infertility (≥1 year of unprotected intercourse without conception) and confirmed clinical varicocele by physical examination and Doppler ultrasound. Exclusion criteria included patients with hormonal abnormalities, genetic disorders, azoospermia, chronic systemic diseases, or those who refused surgery or declined to participate.

The sample size was calculated using the change in sperm DNA fragmentation index (DFI) before and after surgery. Based on published data, a paired t-test with 80% power at $\alpha = 0.05$ indicated a requirement of 22 participants. To account for potential dropouts, the final target was set at 27 participants [13].

Study Variables: The primary outcome variable was the Sperm DNA Fragmentation Index (DFI). Secondary outcome variables included sperm count, sperm motility, and sperm morphology. Baseline characteristics, such as age, BMI, duration of infertility, varicocele grade, and smoking status, were recorded for each participant.

Surgical Procedure: All patients underwent microsurgical varicocelectomy performed by a senior urologist. The procedure was carried out under general or regional anesthesia using a high magnification surgical microscope to ensure accurate ligation of dilated veins while sparing the arteries, lymphatics, and vas deferens. Postoperative care and counseling were provided, and patients were advised to refrain from ejaculation for three days before follow-up semen analysis.

Sample Collection and Analysis: Semen samples were collected through masturbation after 3–5 days of abstinence at baseline (pre-surgery) and six months post-surgery. Semen analysis was performed according to the World Health Organization (WHO) guidelines. The parameters evaluated included:

Sperm count (millions/mL)

Sperm motility (% motile sperm)

Sperm morphology (% normal forms)

Sperm DNA fragmentation was assessed using the sperm chromatin structure assay (SCSA) with flow cytometry. The DFI was calculated as the percentage of sperm with fragmented DNA.

Statistical Analysis: Data were analyzed using SPSS version 25. Descriptive statistics were calculated for baseline characteristics and semen parameters. Continuous variables were presented as mean ± standard deviation (SD). Paired t-tests were used to compare pre- and post-surgery means for semen parameters and DFI. Stratified analyses were performed to assess the impact of age, BMI, duration of infertility, varicocele grade, and smoking status on surgical outcomes. A p-value < 0.05 was considered statistically significant.

Results:

The study included 27 patients with a mean age of **34.93 ± 5.73 years** and a mean body mass index (BMI) of **24.45 ± 3.69 kg/m²**. The mean duration of infertility among the participants was **5.96 ± 2.98 years**.

The comparison of pre- and post-surgery semen parameters and sperm DNA fragmentation index (DFI) in 27 patients revealed significant improvements following microsurgical varicocelectomy. The mean sperm count increased from **24.46 ± 12.04 million/mL** pre-surgery to **38.01 ± 13.88 million/mL** post-surgery (p<0.001p < 0.001p<0.001). Similarly, sperm motility improved significantly, with the mean increasing from **45.40 ± 11.29%** to **51.15 ± 10.46%** (p=0.040p = 0.040p=0.040). Sperm morphology also showed a notable enhancement, with the percentage of normal forms rising from **4.90 ± 1.74%** to **6.40 ± 1.73%** (p=0.002p = 0.002p=0.002). Furthermore, a substantial reduction in sperm DNA fragmentation was observed, as the DFI decreased from **19.37 ± 3.07%** pre-surgery to **9.46 ± 3.22%** post-surgery (p<0.001p < 0.001p<0.001). These findings underscore the effectiveness of microsurgical varicocelectomy in improving semen quality and reducing sperm DNA damage in patients with varicocele-associated infertility. (Table 1)

The stratified analysis showed significant improvements in sperm count for the 25–34 years (p=0.032p = 0.032p=0.032) and 35–39 years (p=0.039p = 0.039p=0.039) age groups, but not for the 40–44 years group (p=0.118p = 0.118p=0.118). Sperm motility improved significantly in the 40–44 years group (p=0.033p = 0.033p=0.033), while sperm morphology increased significantly only in the 25–34 years group (p=0.007p = 0.007p=0.007). Sperm DNA fragmentation index (DFI) decreased significantly across all age groups (p<0.05p < 0.05p<0.05), indicating the procedure's consistent effectiveness in reducing sperm DNA damage. These findings suggest younger patients

benefit more in semen quality improvements, while motility enhancements are more pronounced in older patients. (Table 2)

The stratified analysis based on obesity status revealed differences in the outcomes of microsurgical varicocelectomy. Significant improvements in sperm count (p=0.001p = 0.001p=0.001), sperm morphology (p=0.002p = 0.002p=0.002), and DFI (p=0.000p = 0.000p=0.000) were observed in non-obese patients. In contrast, these parameters did not show statistically significant changes in obese patients, likely due to the small sample size (N=2N = 2N=2). Sperm motility showed a trend toward improvement in non-obese patients (p=0.098p = 0.098p=0.098) but was not significant in either group. These findings suggest that the benefits of microsurgical varicocelectomy may be more pronounced in non-obese patients, potentially due to differences in baseline reproductive health or physiological responses to the surgery. Further research with larger sample sizes is recommended to confirm these observations in obese patients. (Table 3)

The stratified analysis by duration of infertility showed significant improvements in **sperm count** in both groups (1–5 years: p=0.010p = 0.010p=0.010, 6–10 years: p=0.018p = 0.018p=0.018). Significant improvements in **sperm motility** were observed in the 1–5 years group (p=0.039p = 0.039p=0.039), while the 6–10 years group did not show significant changes (p=0.361p = 0.361p=0.361). Improvements in **sperm morphology** were statistically significant in the 6–10 years group (p=0.017p = 0.017p=0.017), but not in the 1–5 years group (p=0.071p = 0.071p=0.071). Both groups showed substantial and significant reductions in **sperm DNA fragmentation index (DFI)** (both p=0.000p = 0.000p=0.000). (Table 4)

The stratified analysis based on varicocele grade revealed significant improvements in sperm count for patients with grade I (p=0.033p = 0.033p=0.033) and grade II (p=0.020p = 0.020p=0.020), while patients with grade III did not show a statistically significant change (p=0.193p = 0.193p=0.193). Sperm motility and morphology showed improvements across all grades, but these changes were not statistically significant (p>0.05p > 0.05p>0.05). Sperm DNA fragmentation index (DFI) demonstrated significant reductions across all grades, with p=0.000p = 0.000p=0.000 for grades I and II, and p=0.012p = 0.012p=0.012 for grade III. This consistent reduction highlights the procedure's effectiveness in reducing sperm DNA damage irrespective of varicocele grade. These findings suggest that microsurgical varicocelectomy has the greatest impact on improving sperm count in patients with lower varicocele grades (I and II). However, the reduction in DFI remains a consistent benefit across all grades. Further research may be needed to explore the less pronounced improvements in higher-grade varicoceles. (Table 5)

The stratified analysis by smoking status revealed significant improvements in sperm count for both smokers (p=0.039p = 0.039p=0.039) and non-smokers (p=0.002p = 0.002p=0.002), with non-smokers showing a slightly larger improvement. Sperm motility did not show statistically significant changes in either group, though smokers demonstrated a trend toward improvement (p=0.102p = 0.102p=0.102). Sperm morphology significantly improved in smokers (p=0.008p = 0.008p=0.008), while the improvement in non-smokers was not statistically significant (p=0.116p = 0.116p=0.116). Both groups exhibited substantial and significant reductions in sperm DNA fragmentation index (DFI) (p=0.000p = 0.000p=0.000), highlighting the consistent effectiveness of microsurgical varicocelectomy in reducing sperm DNA damage. (Table 6)

Table 1: Comparison of Pre- and Post-Surgery Semen Parameters and Sperm DNA Fragmentation Index (DFI)

Variable Pair	**N**	**Pre Mean ± SD**	**Post Mean ± SD**	**p-value**
Sperm Count	27	24.46 ± 12.04	38.01 ± 13.88	0.000

Variable Pair	N	Pre Mean ± SD	Post Mean ± SD	p-value
Sperm Motility	27	45.40 ± 11.29	51.15 ± 10.46	0.040
Sperm Morphology	27	4.90 ± 1.74	6.40 ± 1.73	0.002
Sperm DNA Fragmentation Index (DFI)	27	19.37 ± 3.07	9.46 ± 3.22	0.000

Table 2: Stratified Analysis of Pre- and Post-Surgery Semen Parameters and DNA Fragmentation Index (DFI) by Age Groups

Variable Pair	Age Group	N	Pre Mean ± SD	Post Mean ± SD	p-value
Sperm Count	25-34 years	11	21.68 ± 12.76	35.71 ± 14.86	0.032
	35-39 years	10	27.15 ± 12.66	39.42 ± 11.58	0.039
	40-44 years	6	25.08 ± 10.37	39.90 ± 17.29	0.118
Sperm Motility	25-34 years	11	50.25 ± 7.58	51.31 ± 12.09	0.786
	35-39 years	10	46.12 ± 11.52	52.95 ± 8.84	0.210
	40-44 years	6	35.30 ± 11.60	47.87 ± 10.78	0.033
Sperm Morphology	25-34 years	11	4.70 ± 1.45	6.82 ± 1.78	0.007
	35-39 years	10	4.50 ± 2.09	5.70 ± 1.19	0.089
	40-44 years	6	5.93 ± 1.38	6.78 ± 2.27	0.537
Sperm DNA Fragmentation Index (DFI)	25-34 years	11	20.29 ± 3.30	8.98 ± 3.46	0.000
	35-39 years	10	19.38 ± 3.32	10.05 ± 3.00	0.000
	40-44 years	6	17.65 ± 1.40	9.33 ± 3.53	0.005

Table 3: Stratified Analysis of Pre- and Post-Surgery Semen Parameters and DNA Fragmentation Index (DFI) by Obesity Status

Variable Pair	Obesity Status	N	Pre Mean ± SD	Post Mean ± SD	p-value
Sperm Count	Obese	2	24.05 ± 10.82	36.25 ± 1.20	0.324
	Non-obese	25	24.50 ± 12.33	38.16 ± 14.43	0.001
Sperm Motility	Obese	2	29.85 ± 3.32	47.60 ± 7.50	0.105
	Non-obese	25	46.64 ± 10.77	51.44 ± 10.72	0.098
Sperm Morphology	Obese	2	4.60 ± 1.13	4.75 ± 1.06	0.939
	Non-obese	25	4.92 ± 1.79	6.53 ± 1.72	0.002
Sperm DNA Fragmentation Index (DFI)	Obese	2	21.65 ± 3.61	5.50 ± 0.00	0.100
	Non-obese	25	19.18 ± 3.03	9.77 ± 3.13	0.000

Table 4: Stratified Analysis of Pre- and Post-Surgery Semen Parameters and DNA Fragmentation Index (DFI) by Duration of Infertility

Variable Pair	Duration Group	N	Pre Mean ± SD	Post Mean ± SD	p-value
Sperm Count	1–5 Years	11	20.18 ± 10.54	36.39 ± 14.30	0.010
	6–10 Years	16	27.41 ± 12.43	39.13 ± 13.94	0.018
Sperm Motility	1–5 Years	11	42.20 ± 10.73	51.36 ± 10.18	0.039
	6–10 Years	16	47.59 ± 11.47	51.01 ± 10.97	0.361
Sperm Morphology	1–5 Years	11	4.77 ± 1.48	6.25 ± 1.98	0.071
	6–10 Years	16	4.99 ± 1.94	6.50 ± 1.60	0.017
Sperm DNA Fragmentation Index (DFI)	1–5 Years	11	19.84 ± 3.34	9.95 ± 3.29	0.000
	6–10 Years	16	19.04 ± 2.93	9.12 ± 3.23	0.000

Table 5: Stratified Analysis of Pre- and Post-Surgery Semen Parameters and DNA Fragmentation Index (DFI) by Varicocele Grade

Variable Pair	Varicocele Grade	N	Pre Mean ± SD	Post Mean ± SD	p-value
Sperm Count	I	8	24.20 ± 10.06	40.35 ± 16.69	0.033
	II	14	24.39 ± 12.75	35.31 ± 12.16	0.020
	III	5	25.08 ± 15.35	41.86 ± 15.13	0.193
Sperm Motility	I	8	51.98 ± 8.59	52.31 ± 10.99	0.945
	II	14	43.98 ± 11.75	50.66 ± 9.73	0.105
	III	5	38.84 ± 10.18	50.66 ± 13.71	0.100
Sperm Morphology	I	8	4.95 ± 2.19	6.74 ± 1.45	0.077
	II	14	4.98 ± 1.62	5.92 ± 1.69	0.089
	III	5	4.60 ± 1.61	7.18 ± 2.17	0.120
Sperm DNA Fragmentation Index (DFI)	I	8	18.60 ± 2.62	9.41 ± 3.45	0.000
	II	14	20.00 ± 3.17	9.36 ± 3.15	0.000
	III	5	18.82 ± 3.68	9.80 ± 3.75	0.012

Table 6: Stratified Analysis of Pre- and Post-Surgery Semen Parameters and DNA Fragmentation Index (DFI) by Smoking Status

Variable Pair	Smoking Status	N	Pre Mean ± SD	Post Mean ± SD	p-value
Sperm Count	Yes	13	26.61 ± 12.37	40.02 ± 15.37	0.039
	No	14	22.47 ± 11.82	36.15 ± 12.63	0.002
Sperm Motility	Yes	13	44.38 ± 12.24	51.25 ± 7.78	0.102
	No	14	46.34 ± 10.72	51.06 ± 12.76	0.234
Sperm Morphology	Yes	13	4.82 ± 1.88	6.85 ± 1.71	0.008
	No	14	4.97 ± 1.66	5.97 ± 1.70	0.116
Sperm DNA Fragmentation Index (DFI)	Yes	13	19.70 ± 3.06	9.21 ± 2.89	0.000
	No	14	19.06 ± 3.16	9.69 ± 3.59	0.000

Discussion

The present study evaluates the effects of microsurgical varicocelectomy on sperm parameters and DNA integrity, providing evidence of its efficacy in improving semen quality and reducing sperm DNA damage in men with varicocele-associated infertility. These findings align with existing literature, further substantiating the role of varicocelectomy in enhancing male fertility outcomes.

Reduction in Sperm DNA Fragmentation Index (DFI): Our findings demonstrate a significant reduction in sperm DNA fragmentation following varicocelectomy, with DFI decreasing from 19.37% pre-surgery to 9.46% post-surgery ($p<0.001$). This aligns with Zini et al. [13], who reported a reduction in DFI from 18% to 7% six months post-surgery, emphasizing the surgical correction of oxidative stress as a key mechanism. Cannarella et al. [14] similarly found a significant reduction in DFI (SMD −1.125, $p<0.0001$) across various surgical techniques and testing methods, reinforcing the broad applicability of varicocelectomy in addressing DNA integrity issues. Neto et al. [15] highlighted that the procedure's impact is more pronounced in individuals with elevated baseline DFI levels, which aligns with our findings across different patient subgroups.

Improvements in Semen Parameters: Microsurgical varicocelectomy significantly improved sperm count, motility, and morphology in our study. These results are consistent with those of Kadioglu et al. [16], who reported substantial enhancements in semen concentration, motility, and morphology, alongside reduced DFI. Rochdi et al. [17] observed progressive improvements in sperm concentration and motility over an 18-month follow-up, demonstrating the sustained benefits of the procedure. Similarly, Soetandar et al. [18] reported increases in sperm concentration (+8.23%), motility (+7.17%), and morphology (+0.64%), while Birowo et al. [19] documented improvements in progressive motility (+8.66%) and morphology (+2.73%). These findings collectively support the hypothesis that varicocelectomy mitigates testicular oxidative stress, thereby restoring spermatogenesis.

Age-Specific Outcomes: Our stratified analysis revealed age-related differences in treatment response. Significant improvements in sperm count were observed in patients aged 25–34 years and 35–39 years, while no significant changes were noted in the 40–44 years group. Similar age-dependent trends were reported by Neto et al. [15], who found that younger patients exhibit greater reductions in DFI post-surgery. This suggests that the regenerative capacity of the testicular microenvironment may decline with age, warranting further investigation into age-specific therapeutic strategies.

Impact of Obesity: The stratified analysis based on obesity status revealed that non-obese patients experienced significant improvements in sperm count, morphology, and DFI, whereas these parameters remained unchanged in obese individuals. This finding is consistent with Cannarella et al. [14], who noted that baseline health and oxidative stress levels influence surgical outcomes. The lack of significant changes in obese patients may be attributed to a smaller sample size or underlying metabolic dysfunctions, highlighting the need for tailored interventions in this subgroup

Duration of Infertility: Patients with shorter durations of infertility (1–5 years) exhibited more significant improvements in sperm motility than those with longer durations (6–10 years). This is supported by Rochdi et al. [17], who observed that early intervention yields better outcomes due to less extensive testicular damage. The sustained reduction in DFI across both groups underscores the universal benefit of varicocelectomy in addressing sperm DNA damage, irrespective of infertility duration.

Varicocele Grade: The impact of varicocele grade on surgical outcomes remains equivocal. Our study found significant improvements in sperm count for grades I and II, but not for grade III. Similar findings were reported by Neto et al. [15], who noted a less pronounced effect in higher-grade varicoceles. This could be due to the increased severity of testicular damage in advanced grades, necessitating further research to optimize management strategies for these patients.

Smoking Status: Both smokers and non-smokers benefited from varicocelectomy, with significant reductions in DFI observed in both groups. However, non-smokers demonstrated slightly larger improvements in sperm count, corroborating the findings of Birowo et al. [19]. This suggests that while varicocelectomy mitigates oxidative stress in all patients, smoking cessation may enhance surgical outcomes.

Clinical Implications and Future Directions: The study by Fathi et al. [20] highlighted a significant increase in spontaneous pregnancy rates following varicocelectomy, emphasizing its clinical relevance in improving reproductive outcomes. This underscores the importance of integrating surgical intervention with comprehensive fertility management strategies.

The consistent evidence supporting varicocelectomy's efficacy underscores its role as a cornerstone intervention in managing varicocele-associated infertility. The integration of sperm DNA fragmentation testing into routine clinical practice could refine patient selection and prognostication. Future studies should explore the long-term impact on live birth rates and the potential synergistic benefits of combining varicocelectomy with assisted reproductive techniques, as highlighted by Fathi et al. [20], who demonstrated significant improvements in pregnancy rates post-surgery.

Conclusion: Microsurgical varicocelectomy significantly improves sperm DNA integrity and conventional semen parameters, translating into enhanced fertility outcomes. The findings across multiple studies provide robust evidence for its efficacy, particularly in younger, non-obese patients and those with shorter infertility durations. These insights pave the way for personalized approaches to infertility management, optimizing outcomes for affected individuals

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