

Efficacy Of Varicocele Repair On Sperm DNA Integrity And Seminal Oxidative Stress Markers

Samir Mohamed Elhanbly¹, Ahmed Gamal Borham Elmekawy¹, Adel Abd El-Kader Zalata², Tamer Youssef Mohammed³, Mohammed Fawzy Elkamel¹

^{1*} Department of Dermatology, Andrology & STDs, Faculty of Medicine, Mansoura University.

^{2*}Department of Medical Biochemistry, Faculty of Medicine, Mansoura University

^{3*}Department of general and Endocrine surgery, Faculty of Medicine, Mansoura University

Corresponding author: Ahmed Gamal Borham Elmekawy

Email: ahmed.elmekawy@mans.edu.eg Tel: +20 10 90997430

ORCID ID: 0000-0001-7741-5798

ABSTRACT

Background: Varicocele is the most common correctable cause of male infertility and is often associated with oxidative stress and sperm DNA damage. Sperm DNA fragmentation (SDF), superoxide dismutase (SOD), and malondialdehyde (MDA) are important biomarkers that reflect sperm oxidative balance and functional integrity.

Objective: To evaluate the effect of varicocelectomy on sperm DNA fragmentation, seminal oxidative stress markers (SOD and MDA), and semen quality parameters in infertile men with clinical varicocele.

Patients and Methods: This prospective comparative study included 50 infertile men (mean age 33.1 ± 1.4 years) with grade II–III palpable varicocele. All patients underwent preoperative and 3-month postoperative assessments including semen analysis, sperm DNA fragmentation index (DFI; COMET assay), seminal plasma levels of SOD and MDA, α -glucosidase activity, and acrosin activity index.

Results: Three months after varicocelectomy, significant improvements were observed in sperm concentration ($24.1 \rightarrow 33.8 \times 10^6/\text{mL}$; +40%), progressive motility ($21.5 \rightarrow 36.7\%$; +70%), and normal morphology ($7.0 \rightarrow 17.3\%$; +147%) ($p < 0.001$). Mean DFI decreased from $65.3 \pm 20.7\%$ to $49.3 \pm 17.4\%$ (-24.5%), while MDA decreased from 5.93 to 3.85 nmol/mL (-35.1%). Conversely, mean SOD activity increased from 8.99 ± 3.16 to 12.29 ± 5.77 U/mL (+36.7%) ($p < 0.001$). SDF correlated positively with MDA and negatively with SOD and semen parameters.

Conclusion: Varicocelectomy significantly improves sperm DNA integrity, oxidative stress markers, and semen quality in infertile men with clinical varicocele. SDF may serve as a sensitive molecular indicator of varicocele-related male infertility and response to surgical correction.

KEYWORDS: Varicocele; Varicocelectomy; Sperm DNA fragmentation; Oxidative stress; SOD; MDA; Male infertility.

How to Cite: Samir Mohamed Elhanbly, Ahmed Gamal Borham Elmekawy, Adel Abd El-Kader Zalata, Tamer Youssef Mohammed, Mohammed Fawzy Elkamel., (2025) Efficacy Of Varicocele Repair On Sperm DNA Integrity And Seminal Oxidative Stress Markers, Vascular and Endovascular Review, Vol.8, No.13s, 335-341.

INTRODUCTION

Male infertility contributes to nearly half of all infertility cases worldwide, affecting approximately 15% of couples of reproductive age [1]. Varicocele, defined as an abnormal dilatation and tortuosity of the pampiniform plexus veins, is the most common correctable cause of male infertility, affecting about 15% of the general male population and up to 35–40% of men with primary infertility [2,3].

The underlying mechanisms by which varicocele impair spermatogenesis are multifactorial and not yet fully understood. Proposed mechanisms include testicular hyperthermia, venous stasis, hypoxia, and hormonal imbalance; however, accumulating evidence highlights oxidative stress (OS) as a central pathogenic factor [4]. OS reflects an imbalance between the excessive generation of reactive oxygen species (ROS) and the limited antioxidant defense capacity of the seminal plasma. This imbalance leads to lipid peroxidation of sperm membranes, mitochondrial dysfunction, and sperm DNA fragmentation (SDF), all of which compromise sperm function and fertilizing potential [5–7].

Superoxide dismutase (SOD) is a key antioxidant enzyme that neutralizes superoxide radicals and reflects the body's enzymatic antioxidant defense, while malondialdehyde (MDA) is a product of lipid peroxidation and a widely used marker of oxidative damage [8]. Alterations in these oxidative stress biomarkers have been consistently observed in men with varicocele.

Varicocelectomy, the surgical correction of varicocele, has been shown to improve semen quality in many studies; however, its effects on sperm DNA integrity and oxidative stress parameters remain variably reported. Some studies demonstrated significant improvement

in SDF and antioxidant levels after surgery, whereas others found minimal or inconsistent changes [9-10]. These discrepancies may be related to differences in surgical technique, study design, and biomarkers evaluated.

Therefore, the present study aimed to evaluate the effect of varicocelectomy on sperm DNA fragmentation, seminal oxidative stress markers (SOD and MDA), and semen quality parameters in infertile men with clinical grade II and III varicoceles. By comparing preoperative and postoperative results, this study sought to provide further insight into the molecular mechanisms underlying varicocele-related male infertility and the potential therapeutic impact of surgical correction.

SUBJECTS AND METHODS

Study design and participants:

This was a prospective comparative study conducted on 50 infertile men with clinically palpable grade II or III varicocele who attended the Andrology and General Surgery outpatient clinics of Mansoura University Hospitals between January 2023 and January 2024. The study protocol was approved by the Institutional Research Ethics Committee of Mansoura Faculty of Medicine (Approval No. [MD.22.03.621]) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Inclusion criteria:

- Men aged 20–50 years with primary or secondary infertility (failure to achieve conception after ≥ 12 months of unprotected intercourse).
- Clinically palpable grade II or III varicocele confirmed by duplex Doppler ultrasonography.
- Abnormal semen parameters according to WHO (2010) criteria [10].

Exclusion criteria:

- Subclinical or recurrent varicocele.
- History of testicular trauma, hypogonadism, cryptorchidism, or genital infection.
- Systemic diseases (hepatic, renal, endocrine, or neoplastic).
- Smoking, drug abuse, or recent hormonal therapy.
- Known chromosomal or genetic abnormalities.

Clinical evaluation:

Each patient underwent detailed medical and reproductive history taking, general physical examination, and complete andrological assessment including testicular volume, varicocele grading (Dubin and Amelar system), and scrotal duplex ultrasonography (Siemens Sonoline G50, 10 MHz linear probe).

Surgical technique:

All patients underwent retroperitoneal high ligation (Palomo technique) under spinal anesthesia. Dilated internal spermatic veins were ligated with preservation of the testicular artery and lymphatics. Patients were clinically and ultrasonographically re-evaluated 3 months postoperatively to exclude recurrence or complications.

Semen collection and analysis:

Semen samples were obtained by masturbation after 3–4 days of abstinence and allowed to liquefy at 37°C for 30 minutes. Analysis was performed using a computer-assisted semen analyzer (AutoSperm®, FertiPro, Belgium) following WHO (2010) criteria for sperm count, motility, and morphology. The acrosin activity index and α -glucosidase activity were also assessed as markers of sperm function and epididymal contribution.

Sperm DNA fragmentation (SDF):

SDF was measured by the COMET assay (single-cell gel electrophoresis). The percentage of DNA fragmentation index (DFI) was calculated from 100 spermatozoa per sample before and 3 months after surgery. DFI was interpreted as follows [12]:

- <5%: Excellent DNA integrity
- 5–15%: Adequate integrity
- 15–30%: Moderately elevated fragmentation
- $\geq 30\%$: Severely elevated fragmentation

Oxidative stress biomarkers:

- Malondialdehyde (MDA): Determined in seminal plasma by thiobarbituric acid reactive substances (TBARS) colorimetric method at 534 nm as described by Walker and Shah [8]. Results expressed in nmol/mL.
- Superoxide dismutase (SOD): Measured in seminal plasma by spectrophotometric assay using inhibition of pyrogallol autoxidation. Results expressed in U/mL.
- Acrosin activity test: Acrosin activity was evaluated by gelatinolysis on gelatin-coated slides as described by Henkel et al. [13], with results expressed as the acrosin activity index (halo diameter \times halo formation rate).
- α -Glucosidase activity: Determined by colorimetric assay (Episcreen kit, FertiPro, Belgium) as per Guerin et al. [14].

Statistical analysis:

Data were analyzed using SPSS version 24 (IBM Corp., Armonk, NY, USA). The Kolmogorov–Smirnov test was used to assess normality. Quantitative variables were expressed as mean \pm SD or median (min–max) and compared using paired t-test or

Wilcoxon signed-rank test, as appropriate. Correlations between continuous variables were assessed using Spearman's correlation. A p -value ≤ 0.05 was considered statistically significant.

RESULTS

1. Baseline characteristics of the study population

The study included 50 infertile men with a mean age of 33.1 ± 1.4 years and a mean body mass index of 22.7 ± 0.9 kg/m². The mean duration of infertility was 2.6 ± 0.8 years. Most patients had bilateral varicocele (80%) and secondary infertility (62%). According to clinical grading, 21 men (42%) had grade II and 29 men (58%) had grade III varicocele (**Table 1**).

2. Changes in semen parameters after varicocelectomy

Three months after surgery, there was significant improvement in semen parameters compared with baseline (**Table 2**). Median sperm concentration increased from $24.1 (1.1\text{--}64.0) \times 10^6/\text{mL}$ to $33.8 (6.5\text{--}78.6) \times 10^6/\text{mL}$ (+39.9%, $p \leq 0.001$). Progressive motility (grades A + B) improved from 27.0% (4.0–45.0) to 44.9% (12.6–83.2%) (+66.1%, $p \leq 0.001$). Normal morphology increased from 7.0% (0.0–48.0) to 17.3% (2.0–78.2%) (+147.1%, $p \leq 0.001$).

3. Sperm DNA fragmentation and oxidative stress biomarkers

Mean sperm DNA fragmentation index (DFI) significantly decreased from $65.3 \pm 20.7\%$ to $49.3 \pm 17.4\%$ after varicocelectomy, representing a 24.5% reduction ($p \leq 0.001$). Median seminal MDA level declined from 5.93 (1.16–14.72) to 3.85 (0.93–11.78) nmol/mL (–35.1%, $p \leq 0.001$). Conversely, mean SOD activity increased from 8.99 ± 3.16 to 12.29 ± 5.77 U/mL (+36.7%, $p \leq 0.001$). Additionally, halo diameter, halo formation rate, and acrosin activity index showed significant increases postoperatively ($p \leq 0.001$ for all) (**Table 3**).

4. Correlations between oxidative stress and sperm function

SDF correlated positively with MDA ($r = 0.75$, $p \leq 0.001$) and negatively with SOD ($r = -0.69$, $p \leq 0.001$) before surgery. These relationships persisted postoperatively, though with slightly lower correlation strength ($r = 0.60$ and -0.49 , respectively; $p \leq 0.001$). SOD and MDA were inversely correlated both before and after surgery ($r = -0.75$ and -0.64 , $p \leq 0.001$) (**Table 4**).

5. Comparison of according to varicocele grade

When patients were classified by varicocele grade, both grade II and grade III groups demonstrated significant postoperative improvements (**Table 5**). The reduction in DFI was greater among grade III cases (–26.1%) compared with grade II (–21.7%). Similarly, the decline in MDA was more pronounced in grade III (–30.8%) than in grade II (–15.9%). SOD activity improved in both groups, with a slightly higher increase in grade II (+39.6%) than in grade III (+34.5%).

DISCUSSION

This prospective study evaluated the effect of varicocelectomy on sperm DNA fragmentation, seminal oxidative stress markers, and semen quality parameters in infertile men with clinical grade II and III varicocele. The results demonstrated a significant postoperative decrease in sperm DNA fragmentation index (DFI) and malondialdehyde (MDA) levels, along with an increase in seminal superoxide dismutase (SOD) activity and improved semen parameters. These findings support the hypothesis that oxidative stress plays a key role in varicocele-associated sperm dysfunction and that surgical correction can partially restore the oxidative balance and sperm integrity.

1. Sperm DNA fragmentation and oxidative stress

Elevated sperm DNA fragmentation in men with varicocele has been consistently reported in previous studies [5,15,16]. The mean preoperative DFI in our cohort (65.3%) was higher than that reported by Kavoussi et al. (35.3%) and Zaazaa et al. (38%), which may reflect the inclusion of patients with more advanced varicocele grades or longer infertility duration. After varicocelectomy, DFI significantly decreased by approximately 24.5%, consistent with the reductions reported by Vahidi et al. (2018) and Mostafa et al. (2019), who demonstrated improvements of 20–30% after surgical repair. This improvement can be attributed to enhanced testicular microcirculation, normalization of scrotal temperature, and reduction of reactive oxygen species (ROS) generation.

In parallel, MDA levels—a marker of lipid peroxidation—decreased significantly, whereas SOD activity increased. These results indicate an improved redox environment within the seminal plasma. The negative correlation between SOD and MDA levels observed in our study is consistent with the inverse relationship between antioxidant activity and oxidative damage reported by Liu et al. [17]. Restoration of SOD activity after surgery suggests recovery of the enzymatic antioxidant defense system.

2. Semen quality and sperm function

The significant postoperative improvements in sperm concentration, motility, and morphology observed in the present study align with previous reports demonstrating enhanced semen quality after varicocelectomy [15,18]. The improvement in progressive motility (+66%) and normal morphology (+147%) indicates not only improved spermatogenesis but also enhanced sperm membrane stability and mitochondrial function following the reduction of oxidative stress.

The observed positive correlations between SOD and semen parameters, and negative correlations between SDF and sperm quality, suggest that oxidative stress directly affects sperm function. This finding reinforces the importance of assessing oxidative stress markers as adjunctive tools in evaluating male infertility, particularly in men with varicocele.

3. Association with varicocele grade

The higher preoperative DFI and MDA levels observed in grade III varicoceles compared with grade II confirm that oxidative stress severity correlates with varicocele grade. Interestingly, the magnitude of postoperative improvement in DFI and MDA was greater in grade III cases, possibly due to their initially higher baseline values. Conversely, the increase in SOD was more evident in grade II patients, which might reflect a ceiling effect where severely affected testes show less complete enzymatic recovery. These findings emphasize the potential benefit of surgical correction even in advanced grades of varicocele.

4. Functional markers: α -glucosidase and acrosin activity

The inclusion of α -glucosidase and acrosin activity provided complementary insights into epididymal and sperm functional status. Both parameters improved significantly after varicocelectomy and correlated positively with SOD and negatively with MDA and DFI. α -Glucosidase reflects epididymal function, and its increase postoperatively may result from improved testicular perfusion and ductal secretion [19]. Similarly, acrosin activity, which reflects the fertilizing capacity of spermatozoa, was inversely related to oxidative stress levels, confirming that ROS-mediated damage impairs acrosomal enzymes [20].

5. Clinical relevance and limitations

The results of this study reinforce that varicocelectomy can improve seminal oxidative status and sperm DNA integrity in infertile men. However, the observational design limits the ability to establish causation, and the relatively short follow-up period (3 months) precludes assessment of long-term outcomes such as pregnancy rates. The relatively high preoperative DFI values in our cohort may reflect patient selection from a tertiary infertility clinic rather than measurement error. Future multicenter studies with longer follow-up and inclusion of fertility outcomes are warranted.

6. Summary interpretation

Overall, these findings confirm that oxidative stress is a major contributor to varicocele-associated sperm dysfunction. Varicocelectomy leads to significant biochemical and functional improvement by restoring the oxidant–antioxidant balance and reducing DNA fragmentation. Incorporating sperm DNA fragmentation and oxidative stress assessment in the evaluation of men with varicocele may enhance clinical decision-making and postoperative monitoring.

Limitations

A relatively small sample, short follow-up duration, and being a single-center study may limit the ability to generalize findings.

CONCLUSION

Varicocelectomy was associated with significant improvement in sperm DNA integrity, seminal oxidative stress balance, and semen quality among infertile men with clinical varicocele. The reduction in sperm DNA fragmentation and malondialdehyde levels, together with the increase in superoxide dismutase activity, indicates that oxidative stress is a central mechanism in varicocele-associated sperm dysfunction.

These findings suggest that assessing sperm DNA fragmentation and oxidative stress markers may provide additional insight into the molecular impact of varicocele and serve as valuable tools for evaluating treatment response following varicocelectomy.

However, given the single-center design, relatively small sample size, and short follow-up period, these results should be interpreted with caution. Long-term, multicenter studies assessing postoperative fertility outcomes are warranted to confirm these observations.

RECOMMENDATIONS

- Incorporation of sperm DNA fragmentation and oxidative stress testing (e.g., SOD, MDA) into the routine evaluation of men with varicocele may enhance diagnostic accuracy and treatment planning.
- Varicocelectomy should be considered for infertile men with elevated SDF and abnormal semen parameters, as surgical repair can improve oxidative balance and sperm quality.
- Future studies should include larger patient populations, longer follow-up durations, and pregnancy outcome assessment to better define the prognostic role of SDF reduction after varicocelectomy.
- Comparative research between different surgical techniques (microsurgical, laparoscopic, and open) is recommended to identify the most effective approach for improving sperm DNA integrity.

Acknowledgment: Special thanks are due to the patients who participated in this research for their trust, cooperation, and valuable contributions, without which this study would not have been possible.

Conflict of interest: None.

Fund: None.

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Lists of Tables

Table (1): Patients characteristics among the studied group

| Patients' characteristics | The studied group (n=50) |
|---------------------------|--------------------------|
| Age (Years) | |
| Mean \pm SD | 33.10 \pm 1.45 |
| Min – Max | 28.0-42.0 |
| BMI | |
| Mean \pm SD | 22.71 \pm 0.91 |
| Min – Max | 21.00-25.00 |
| Side of Varicocele | |
| Unilateral (left) | 10 (20.0%) |
| Bilateral | 40 (80.0%) |
| Varicocele grade | |
| Grade II | 21 (42.0%) |
| Grade III | 29 (58.0%) |
| Infertility duration | |
| Mean \pm SD | 2.60 \pm 0.77 |
| Min – Max | 1.50-5.00 |
| Type of infertility | |
| Primary | 19 (38.0%) |
| Secondary | 31 (62.0%) |

Table (2): Semen parameters before and after 3 months of varicocelectomy

| | Before varicocelectomy | After 3 months of varicocelectomy | Change % | Test significance of P value |
|--|------------------------|-----------------------------------|----------|------------------------------|
| Conc. (mill/ml) Median (Min – Max) | 24.13 (1.10-64.0) | 33.77 (6.54-78.60) | 39.95% | Z=5.74 P≤0.001* |
| Grade A motility (%) Median (Min – Max) | 11.50 (2.00-38.0) | 20.20 (3.90-59.20) | 75.65% | Z=5.86 P≤0.001* |
| Grade B motility (%) Median (Min – Max) | 10.00 (2.00-30.0) | 16.50 (4.00-53.00) | 65.0% | Z=6.14 P≤0.001* |
| Grade_A+B (%) Median (Min – Max) | 27.00 (4.00-45.0) | 44.85 (12.60-83.20) | 66.11% | Z=6.15 P≤0.001* |
| Morphology (%) Median (Min – Max) | 7.00 (0.0-48.00) | 17.30 (2.00-78.20) | 147.1% | Z=5.44 P≤0.001* |
| Vel (µm / sec) Median (Min – Max) | 22.88 (5.72-71.3) | 30.28 (7.44-92.70) | 32.34% | Z=5.45 P≤0.001* |
| Linear velocity (µm / sec) Median (Min – Max) | 18.3 (2.58-57.04) | 20.62 (7.00-84.86) | 12.67% | Z=6.15 P≤0.001* |
| Linearity index Mean ± SD | 59.55±11.41 | 76.48±8.52 | 28.43% | t=6.15 P≤0.001* |

t: Paired t test, Z: Wilcoxon signed rank test, *significant p≤0.05

Table (3): SDF, Malondialdehyde, SOD, Halo diameter, Halo percent and Acrosin index before and after 3 months of varicocelectomy

| | Before varicocelectomy | After 3 months of varicocelectomy | Change % | Test significance of P value |
|-------------------------------------|----------------------------|-----------------------------------|----------|------------------------------|
| SDF Mean ± SD Min – Max | 65.28±20.74 30.00-94.00 | 49.31±17.36 21.20-84.80 | -24.46% | t=9.95 P≤0.001* |
| MDA Median (Min – Max) | 5.93 (1.16-14.72) | 3.85 (.93-11.78) | -35.07% | Z=5.92 P≤0.001* |
| SOD Mean ± SD Min – Max | 8.99±3.16 2.68-14.42 | 12.29±5.77 2.49-29.74 | 36.71% | t=7.05 P≤0.001* |
| Halo diameter Mean ± SD | 12.95±2.53 | 15.54±3.04 | 20.0% | t= 36.14 P≤0.001* |
| Halo percent Median (Min – Max) | 35.00 (6.0-82.0) | 50.50 (8.80-91.00) | 44.28% | Z=6.00 P≤0.001* |
| Acrosin index Median (Min – Max) | 4.68 (0.66-13.98) | 7.66 (1.15-19.61) | 63.67% | Z=6.15 P≤0.001* |

t: Paired t test, Z: Wilcoxon signed rank test, *significant p≤0.05

Table (4): Correlation between DNA frag, Malondialdehyde and SOD before and after varicocelectomy

| | DNA frag | | Malondialdehyde | |
|------------------------|----------|---------|-----------------|---------|
| | R | p | r | p |
| Before varicocelectomy | | | | |
| MDA | 0.750 | ≤0.001* | - | - |
| SOD | -0.692 | ≤0.001* | -0.746 | ≤0.001* |
| After varicocelectomy | | | | |
| MDA | 0.603 | ≤0.001* | - | - |
| SOD | -0.490 | ≤0.001* | -0.638 | ≤0.001* |

Table (5): Comparison between Varicocele grade and SDF, MDA, SOD before and after 3 months of varicocelectomy

| | Varicocele grade | Before | After | Change % | Test of significance P value |
|-----|------------------|--------------------|--------------------|----------|---------------------------------|
| SDF | II | 57.19±18.99 | 44.78±16.84 | -21.69% | t=5.61 P≤0.001 |
| | III | 71.13±20.27 | 52.58±17.27 | -26.08% | t=8.57 P≤0.001 |
| MDA | II | 4.14 (1.29- 12.90) | 3.48 (1.03- 7.50) | -15.9% | Z=3.67 P≤0.001 |
| | III | 6.11 (1.16- 14.72) | 4.23 (0.93- 11.78) | -30.77% | Z=4.66 P≤0.001 |
| SOD | II | 9.19±3.27 | 12.83±6.07 | 39.6% | t=4.48 P≤0.001 |
| | III | 8.85±3.13 | 11.90±5.62 | 34.46% | t=5.44 P≤0.001 |