Effect of varicocelectomy on detailed sperm morphology parameters
An observational retrospective clinical cohort study

Mehmet Yaris, MD* & Rift Kılınç, MD

Abstract
Although many studies suggest that varicocelectomy leads to improvement in semen parameters and morphology, its clinical efficacy remains controversial. The detailed morphological parameters described in the World Health Organization guidelines are important in terms of showing the effect of microsurgical subinguinal varicocelectomy on morphological changes.

An observational, retrospective clinical cohort study was conducted with patients followed up from January 2018 to August 2021. This study included the data of 79 patients who met the criterion of undergoing at least 2 detailed morphological evaluations before and after surgery. All operations were performed by the same surgical team using the microsurgical subinguinal varicocelectomy technique.

The mean age of the patients was 30.25 years. Of the patients, 63 underwent left-sided varicocelectomy and 16 underwent bilateral surgery. The sperm analysis revealed statistically significant increases in sperm volume ($P = .006$), sperm concentration ($P = .003$), total sperm count ($P = .001$), progressive sperm motility ($P < .001$), and normal morphology ($P < .001$). In the detailed morphological evaluation, except for the elongated head anomaly ($P = .037$), no other statistically significant changes were found in relation to sperm head, tail, and neck anomalies after surgery.

This study makes an important contribution to the literature, being the first to use the subinguinal microscopic varicocelectomy technique in detailed morphological semen evaluation. We consider that detailed morphology examination in the selection and treatment of infertile patients may be useful in evaluating the efficacy of varicocelectomy.

Abbreviations: MSV = microsurgical subinguinal varicocelectomy, WHO = World Health Organization.

Keywords: microsurgical subinguinal varicocelectomy, sperm morphology, varicocele

1. Introduction
Infertility is defined as the inability of couples to conceive despite having engaged in 1 year of unprotected sexual intercourse. [1] According to the World Health Organization (WHO) (2002), approximately 15% of the global population is affected by infertility, and male infertility is responsible for 50% of all cases. [2] Male infertility is associated with various parameters, such as congenital factors, varicoceles, testicular cancer, hypogonadism, infection, autoimmune diseases, systemic diseases, genetic abnormalities, and undescended testis. A varicocele is associated with decreased sperm quality and infertility and is one of the most common correctable causes of male infertility. [3] The prevalence of a clinically significant varicocele varies between 5% and 20%. It affects 19% to 41% of men with primary infertility and 45% to 81% of those with secondary fertility. [4, 5]

Many studies have investigated the efficacy of varicocelectomy on improvement in semen parameters, and several authors have found a significant improvement in semen quality after this surgery. [6–8] Although many meta-analyses have shown that all techniques used for varicocele treatment improve sperm parameters, microsurgical varicocelectomy (MSV) is the optimal technique due to its lower rate of complications, such as hydrocele and varicocele recurrence. [9–11] According to a meta-analysis by Cayan et al, [12] the rate of hydroceles was significantly lower in MSV (0.44%) than in laparoscopic varicocelectomy (2.84%). Similarly, the rate of varicocele recurrence was found to be significantly lower in MSV (1.05%) than in laparoscopic varicocelectomy (4.3%) and...
radiological embolization (12.7%). These findings were confirmed by further comparative studies.\cite{13}

Schauer et al.\cite{14} reviewed 14 studies and highlighted a significant improvement in both sperm count and motility after surgical varicocelectomy. Similarly, Kim et al.\cite{15} published a meta-analysis examining 7 studies that reported a significant increase in progressive sperm motility, with no effect on sperm count and morphology. In a broad sense, it can be assumed that surgical varicocelectomy improves semen parameters in only a subset of patients and shows its beneficial effects primarily on semen quality rather than sperm count. However, the vast majority of studies on this subject have not reported an improvement in sperm morphology. The spectrum of sperm morphological anomalies described in the WHO guidelines is broad and detailed, with these parameters often being reported as binary variants (i.e., presence or absence of morphological anomalies) in clinical practice. In a recent study by Morini et al.\cite{15} the effect of varicocelectomy on detailed morphological parameters was investigated. However, in that study, more than 1 surgical method was used and more than 1 surgeon performed the operations. In light of this information, our study was designed to evaluate the effects of microsurgical subinguinal varicocelectomy repair of varicoceles on detailed morphological microscopic sperm characteristics in a large, homogeneous patient group followed up in the same clinical center.

2. Materials and methods

For this retrospective study, local ethics committee approval was obtained from Gazi Yaşargil Training and Research Hospital (approval date: 11.06.2021, number: 802), and all the patients provided informed consent. An observational, retrospective clinical cohort study was conducted with patients followed up from January 2018 to August 2021. In this study, all the patients with varicoceles were operated on by the same surgeon using the microsurgical subinguinal varicocelectomy technique. The files of 165 patients who underwent varicocelectomy during this period were reviewed. The data of 79 patients who met the following criteria were included in the study: diagnosis of varicoceles on ultrasound and physical examination, presence of abnormal sperm parameters or infertility, and availability of semen analysis results obtained from at least 2 detailed morphological evaluations in the preoperative and postoperative periods. Patients who had an endocrine disease that could affect sperm count, those using antioxidant drugs, and those with a history of previous urogenital surgery or any infertility treatment were not included in the study. The degree of especially varicoceles was evaluated under resting conditions after the Valsalva maneuver and graded according to Sarteschi 5-item scale.\cite{16} In addition, study data were obtained from semen samples before and at 3 to 12 months after surgical varicocele repair at each examination depending on clinical practice.

2.1. Microscopic subinguinal varicocelectomy technique

An approximately 2.5 cm incision was made below the external inguinal ring and above the ramus pubis. The surrounding tissue was released under Scarpa’s fascia and the cord was reached. The cord was raised above skin level usingatraumatic Babcock forceps. The cord was suspended, and the external spermatic veins were detected by thorough ventilation, and then ligated and cut on both sides. The spermatic fascia was opened using monopolar cautery. At this stage, a microscopic image (6–10 times magnification) was obtained with a surgical microscope (Leica M525 F20). The vas deferens, artery(s), and lymphatic vessels in the cord were preserved as much as possible. All the veins were suspended with microforceps and dissectors, and ligated on both sides using 3/0 or 4/0 free silk sutures. Homeostasis was checked, and the layers were closed on the anatomical plane.

2.2. Semen analysis

Semen analysis was performed according to the 5th version of the WHO guidelines.\cite{17} Semen parameters were analyzed for sperm volume, count, motility, and morphology. Microscopic and morphological sperm evaluation was performed in 3 consecutive steps. After evaluating sperm count and motility, the sample fixed with alcohol for 3 minutes was kept in Giemsa stain 10 minutes. After washing and drying, a morphological examination was performed at 100 magnification under a microscope (Olympus CX43) with immersion oil. According to the WHO recommendations, sperm morphology was evaluated in detail considering the head, midpiece, and tail. In particular, this evaluation specifically includes the possible presence of an elongated, pyriform, duplicated, irregular, microcephaly, macrocephaly, duplicated, and/or vacuolated sperm head among head anomalies. The anomalies of the neck and midpiece were defined based on the presence of the asymmetrical or angled placement of the midpiece to the head and/or excess of abnormal cytoplasm greater than one-third of the head size. Tail anomalies were reported in the case of a short, absent, curled, and/or double tail. Although WHO has defined a detailed morphological microscopic sperm evaluation, this is not routinely used in clinical practice.

2.3. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 17 (USA). The conformity of the variables to the normal distribution was examined with histogram graphs and the Kolmogorov–Smirnov test. Mean, standard deviation, and median values were used when presenting descriptive statistics. Categorical variables were compared with the Pearson chi-square test. The Mann–Whitney U test was used when evaluating non-normally distributed (non-parametric) variables between 2 groups, and the Kruskal–Wallis test was conducted for the evaluation between more than 2 groups. Changes in the measured values were examined using the Wilcoxon test. Results with a P value below .05 were considered as statistically significant.

3. Results

Of the 79 patients included in the study, 37 were >30 years and 42 were ≤30 years. The mean age of the patients was 30.25 years. Of all the patients, 63 underwent varicocelectomy on the left side and 16 underwent bilateral surgery. The physical examination revealed grade 1 varicoceles in 5 patients, grade 2 varicoceles in 59, and grade 3 varicoceles in 15. The vessel diameter was ≤3 mm in 41 patients and >3 mm in 38 patients (Table 1).

After the operation, there were 49 (62.03%) patients with an increase in sperm concentration, 52 (65.82%) with an increase in total sperm count, 53 (67.09%) with an increase in advanced
sperm motility, and 41 (51.9%) with an increase in normal sperm morphology. When changes in the sperm analysis values were examined, they were statistically significant for sperm volume ($P = .006$), sperm concentration ($P = .003$), total sperm count ($P = .001$), progressive sperm motility ($P < .001$), non-progressive sperm motility ($P = .001$), immotile sperm count ($P = .011$), and normal morphology ($P < .001$). The detailed morphological evaluation revealed that except for the elongated head anomaly ($P = .037$), there were no other statistically significant changes in relation to the sperm head, tail, and neck anomalies after surgery (Table 2).

The amount of changes in the measured values was compared according to age groups, varicoceles grades determined during the physical examination, and Doppler ultrasound (US) vessel diameter, and no significant result was found. When the amount of changes in the measured values was compared according to the side of operation, the values of sperms with amorphous heads increased in the patients that had undergone bilateral varicocelectomy, while it decreased in those that underwent surgery on the left side (Table 3).

The patients with an increase in sperm concentration, total sperm count, progressive sperm motility, and normal morphology were compared according to age groups, and the rate of those with an increase in total sperm count at the age of 30 years and younger was found to be higher than those over 30 years ($P = .038$). In other words, total sperm count increased in a greater proportion of patients under 30 years (Table 4). When the same parameters were compared in terms of varicocele grade and vessel diameter in Doppler US, no significant difference was found.

4. Discussion

Although many studies argue that varicocelectomy has positive effects on sperm parameters and morphology, some still argue that it is ineffective, and therefore this issue remains controversial. Varicocelectomy appears to have no effect on morphology parameters in some studies, indicating the need for a different perspective in patient selection for treatment. Previously, Morini et al. [5] evaluated detailed morphology parameters but they used more than 1 surgeon and different surgical techniques in their study. In contrast, in the current study, the microscopic subinguinal surgical technique was used by a single surgeon, and morphology results were evaluated in detail.

Guidelines recommend that varicocelectomy should be performed with the indication of infertility, as evidenced by abnormal sperm parameters, including sperm concentration, motility, and morphology. This surgery is not recommended in infertile patients with normal semen parameters and in those with subclinical varicoceles. [18] There are many studies showing that MSV has positive effects on sperm parameters. In a prospective, randomized, controlled study, Abdel-Meguid et al. [19] observed that the total sperm count, sperm concentration, sperm motility, and normal morphology ratio significantly improved in patients who underwent MSV compared to the control group. Similarly, Leung et al. [20] reported a statistically significant improvement in the mean sperm concentration (from 12 to 23 million/mL), motility (from 26% to 32%), and normal

| Table 1 |
| --- |
| Clinical characteristics of the patients. |
|  |
| n | % |
| --- | --- |
| Age |
| $\leq 30$ years | 42 | 53.16 |
| $>30$ years | 37 | 46.84 |
| Operation side |
| Sol | 63 | 79.75 |
| Bilateral | 16 | 20.25 |
| Varicocele grade |
| Grade 1 | 5 | 6.33 |
| Grade 2 | 59 | 74.68 |
| Grade 3 | 15 | 18.99 |
| Vessel diameter on Doppler ultrasound |
| $\leq 3$ mm | 41 | 51.90 |
| $>3$ mm | 38 | 48.10 |

The amount of changes in the measured values was compared according to age groups, varicoceles grades determined during the physical examination, and Doppler ultrasound (US) vessel diameter, and no significant result was found. When the amount of changes in the measured values was compared according to the side of operation, the values of sperms with amorphous heads increased in the patients that had undergone bilateral varicocelectomy, while it decreased in those that underwent surgery on the left side (Table 3).

| Table 2 |
| Changes in preoperative sperm parameters after surgery. |
| --- |
| Preoperative | Postoperative | Difference |
| --- | --- | --- |
| Mean | SD | Median | Mean | SD | Median | Mean | SD | Median | P |
| Volume | 2.64 | ±1.01 | 2.50 | 2.94 | ±1.04 | 3.00 | 0.30 | ±0.93 | 0.20 | .006 |
| Concentration | 24.36 | ±20.48 | 18.00 | 30.56 | ±19.09 | 30.00 | 6.20 | ±19.94 | 6.00 | .003 |
| Total count | 62.05 | ±62.11 | 42.50 | 82.60 | ±50.84 | 75.00 | 20.55 | ±60.26 | 23.00 | .001 |
| Progressive motility | 34.24 | ±11.55 | 31.00 | 42.98 | ±18.26 | 47.00 | 8.74 | ±18.74 | 12.00 | <.001 |
| Non-progressive motility | 21.56 | ±10.24 | 21.00 | 17.04 | ±8.47 | 17.00 | -4.52 | ±12.91 | -5.00 | .001 |
| Immotile | 44.04 | ±14.54 | 43.00 | 39.09 | ±15.74 | 36.00 | -4.95 | ±18.93 | -6.00 | .011 |
| Normal morphology | 3.14 | ±2.06 | 3.00 | 4.77 | ±2.93 | 4.00 | 1.63 | ±3.48 | 1.00 | <.001 |
| Amorphous head | 21.73 | ±12.98 | 22.00 | 21.04 | ±13.31 | 20.00 | -0.70 | ±13.09 | 0.00 | .875 |
| Macrocephaly | 8.90 | ±10.51 | 3.00 | 10.90 | ±11.59 | 6.00 | 2.00 | ±12.57 | 0.00 | .170 |
| Microcephaly | 5.52 | ±8.42 | 0.00 | 5.00 | ±8.75 | 0.00 | -0.52 | ±9.13 | 0.00 | .655 |
| Elongated head | 7.09 | ±11.24 | 0.00 | 4.32 | ±6.92 | 0.00 | -2.77 | ±10.50 | 0.00 | .037 |
| Tail anomaly | 19.44 | ±14.45 | 20.00 | 20.56 | ±12.56 | 20.00 | 1.11 | ±16.13 | 0.00 | .523 |
| Duplicated head | 1.38 | ±4.44 | 0.00 | 0.76 | ±1.92 | 0.00 | -0.62 | ±4.72 | 0.00 | .525 |
| Neck anomaly | 14.91 | ±10.48 | 13.00 | 16.63 | ±9.32 | 18.00 | 1.72 | ±13.11 | 2.00 | .243 |
| Cytoplasmic droplet | 6.34 | ±7.49 | 6.00 | 6.55 | ±7.14 | 4.00 | 0.13 | ±9.86 | 0.00 | .616 |
| Detached head | 1.99 | ±3.88 | 0.00 | 1.43 | ±2.89 | 0.00 | -0.56 | ±4.07 | 0.00 | .275 |
| Pin head | 4.82 | ±6.42 | 2.00 | 5.81 | ±6.86 | 4.00 | 0.99 | ±7.54 | 0.00 | .241 |

Wilcoxon test, SD = standard deviation.
Comparing preoperative and postoperative changes in sperm parameters between the left-sided and bilateral varicocelectomy groups.

|                      | Laterality |                      |                      |
|----------------------|------------|----------------------|----------------------|
|                      | Left       | Bilateral            |                      |
|                      | Mean       | SD       | Median | Mean       | SD       | Median | P     |
| Volume               | 0.32 ± 0.96| 0.20    |        | 0.24 ± 0.80| 0.20    |        | 802   |
| Concentration        | 6.90 ± 3.78| 6.00    |        | 3.44 ± 11.91| 0.00    |        | 373   |
| Total count          | 21.92 ± 5.24| 25.00  |        | 15.16 ± 43.51| 14.00   |        | 479   |
| Progressive motility | 8.05 ± 1.34| 12.00  |        | 11.44 ± 19.72| 11.50   |        | 335   |
| Non-progressive motility | −4.37 ± 13.14| −5.00 |        | −5.13 ± 12.34| −4.50   |        | 956   |
| Immotile             | −4.44 ± 19.18| −5.00 |        | −6.94 ± 18.35| −13.50  |        | 424   |
| Normal morphology    | 1.79 ± 3.63| 1.00    |        | 1.00 ± 2.78 | 0.50    |        | 471   |
| Amorphous head       | −2.71 ± 13.43| −2.00 |        | 7.25 ± 7.78 | 8.50    |        | 0.003 |
| Macrocephaly         | 2.37 ± 13.00| 0.00   |        | 0.56 ± 11.00| 0.00    |        | 547   |
| Microcephaly         | −0.32 ± 9.78| 0.00   |        | −1.31 ± 6.14 | 0.00    |        | 589   |
| Elongated head       | −2.81 ± 10.64| 0.00  |        | −2.63 ± 10.29| 0.00    |        | 567   |
| Tail anomaly         | 1.83 ± 15.54| 0.00   |        | −1.69 ± 18.54| −7.00   |        | 229   |
| Duplicated head      | −0.78 ± 5.24| 0.00   |        | 0.00 ± 1.46  | 0.00    |        | 821   |
| Neck anomaly         | 2.03 ± 12.98| 0.00   |        | 0.50 ± 13.98| 2.50    |        | 859   |
| Cytoplasmic droplet  | 0.83 ± 10.12| 0.00   |        | −2.80 ± 8.33 | −2.00   |        | 170   |
| Detached head        | −0.21 ± 3.88| 0.00   |        | −1.94 ± 4.63 | .00     |        | 398   |
| Pin head             | 0.87 ± 7.82 | 0.00   |        | 1.44 ± 6.53 | 1.00    |        | 777   |

SD = standard deviation.

Table 3

Comparison of patients with improved sperm parameters after surgery according to age.

|                      | ≤30 years | >30 years |
|----------------------|-----------|-----------|
|                      | n   | %   | n   | %   | P    |
| Concentration        | 27  | 64.29 | 22  | 59.46 | .659 |
| Total count          | 32  | 76.19 | 20  | 54.05 | .038 |
| Progressive motility | 26  | 61.90 | 27  | 72.97 | .296 |
| Normal morphology    | 24  | 57.14 | 17  | 45.95 | .320 |

In a meta-analysis evaluating 17 studies, a significant improvement was detected in sperm parameters after MSV.[21] Consistent with these results, in our study, there were significant increases in sperm parameters. Although a detailed microscopic morphological examination is not widely applied in both routine clinical practice and research, microscopic sperm changes have been described in patients with varicoceles.[22]

A more detailed microscopic morphological sperm evaluation showed a decrease in cell abnormalities, although sperm morphology appeared unaffected by surgical correction when reported as binary variants (i.e., normal or abnormal).[5] In our study, there was a significant increase in normal sperm morphology and a significant decrease in abnormal morphology after varicocelectomy. Our detailed morphological examination showed a significant decrease only in the rate of elongated head anomalies.

Morini et al.[5] demonstrated microstructural sperm recovery sustained by a reduction in the head (i.e., macrocephaly, microcephaly, and cytoplasmic appendix) and tail (i.e., agenesis and coiled form) anomalies after surgery. It is considered that the difference of these results from our study is due to the different surgical techniques used and 1 surgeon performing all the operations in our sample. In the current study, there was a significant increase in normal morphology, but a statistically significant decrease was found only in the elongated head anomaly in the detailed examination. Therefore, our results confirm the potential of varicocele treatment in improving semen quality, highlighting a new parameter that can measure this ability. Furthermore, our study also demonstrates potential semen recovery after surgical varicocele repair with a specific new focus on sperm morphology.

In a meta-analysis in which only subclinical varicoceles were evaluated and a significant increase was observed in motility.[23] Although our sample did not include any patient with a subclinical varicocele, we found no significant difference between the groups formed based on the vessel diameter being ≤3 or >3 mm. We also evaluated whether age was associated with postoperative seminal recovery. In our study, all 4 factors evaluated in seminal analysis showed significant improvement after varicocelectomy. Similarly, in a study by Al Bakri et al.[27] the mean sperm count significantly increased at 3 to 6 months after varicocelectomy. However, the authors did not detect a significant improvement in terms of semen volume, motile sperm count, sperm count, and total sperm motility after surgery.[27] In another study conducted by Perimenis et al.[28] patients with grade 1 and 2 varicoceles were shown to have improved motility, morphology, and sperm concentration at 6 months after surgery. In patients with grade 3 varicoceles, only morphology and sperm concentration improved, with no significant change in sperm motility. Several studies, including recently published meta-analyses have found that varicoceles have a significant effect on semen parameters, such as number, motility, and morphology.[29]

The major limitations of our study are its retrospective design and the small number of patients. Another important limitations concern the missing birth rates and the homogeneous nature of...
the patient group operated in a single center. However, it is significant in terms of being the first study in which a single surgeon performed all the operations using only the subinguinal microscopic varicocelectomy technique and a detailed morphological semen evaluation was undertaken. We consider that detailed morphology examination in the selection and treatment of infertile patients may be useful in evaluating the efficacy of varicocelectomy.

5. Conclusion

Although the effects of varicocelectomy on sperm morphology remain controversial, we found positive effects in our study. It is possible to provide a better understanding of these effects by evaluating detailed sperm morphology. In our study, a significant change after MSV was only observed in the elongated sperm head ratio among abnormal sperm morphology parameters. Future studies with a larger number of cases can better evaluate the clinical outcome of morphological improvement.

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Author contributions

Conceptualization: Mehmet Yaris¸.
Data curation: Mahir Kartalmis, Rifaat Kilinc.
Funding acquisition: Mehmet Yaris¸.
Methodology: Mehmet Yaris¸.
Project administration: Mehmet Yaris¸, Rifaat Kilinc.
Resources: Mehmet Yaris¸.
Supervision: Mehmet Karabakan.
Supervision: Mehmet Yaris¸.
Writing – original draft: Mehmet Yaris¸.
Writing – review & editing: Mehmet Karabakan, Mehmet Yaris¸.

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