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ORIGINAL ARTICLE

The influence of antisperm antibodies, intratesticular haemodynamics and the surgical approach to varicocelectomy on seminal variables

Ahmed M. Al-Adl [a,*], Tarek El-Karamany [a], Hesham Issa [b], Mohamed Zaazaa [a]

[a] *Urology Department, Benha Faculty of Medicine, Benha, Egypt*
[b] *Clinical Pathology Department, Benha Faculty of Medicine, Benha, Egypt*

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KEYWORDS

Varicocele;
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ABBREVIATIONS

ASAs, anti-sperm antibodies;
BTB, blood–testis barrier;
PSV, peak systolic velocity;
EDV, end diastolic velocity;
RI, resistive index;
PI, pulsatility index;

Abstract ***Objective:*** To evaluate the effect of antisperm antibodies (ASAs), hormonal levels, intratesticular haemodynamics and the surgical approach on the outcomes of varicocelectomy in infertile men, as assessed by seminal variables.

Patients and methods: In a prospective case-series study, 82 infertile men with varicocele (35 left and 47 bilateral) were evaluated. The preoperative assessment included a physical examination, semen analysis, assessment of ASAs in seminal plasma, hormonal levels (follicle-stimulating hormone (FSH), luteinising hormone and testosterone), and scrotal colour Doppler ultrasonography (CDUS) to measure the peak systolic velocity (PSV), end diastolic velocity (EDV), resistive index (RI) and pulsatility index. Patients were scheduled for varicocelectomy, with high ligation (Palomo) used in 40 patients (18, 45%, with left and 22, 55%, with bilateral varicocele), or an inguinal approach (Ivanissivich) with loupe magnification used in 42 (17, 40%, with left and 25, 60%, with bilateral varicocele). The men were reassessed at ⩾3 months after surgery and according to the improvement in seminal variables (expressed as a ⩾50% increase in total motile sperm count, TMSC), patients were further categorised into 'improved' or 'unimproved'. Binary logistic regression analysis was used to investigate the predictors of improvement.

Results: Before surgery the ASAs were positive in 17 men (21%). There was no significant difference between the right and left sides in intratesticular

* Corresponding author. Tel.: +20 133237560, +20 966533315415.
E-mail address: ahmed.aladl@fmed.bu.edu.eg (A.M. Al-Adl).

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BTV, bilateral testicular volume;
CDUS, colour Doppler ultrasonography;
TMSC, total motile sperm count;
LH, luteinising hormone

haemodynamics. The TMSC was improved in 52 (63%) patients who had a significant improvement in the haemodynamic variables. Intratesticular haemodynamics, serum FSH and testosterone levels differed significantly between the improved and unimproved patients. Positivity for ASAs, the surgical approach and laterality of the varicocele were not significantly different, although the ASA-positive cases were characterised by a significant decrease in motility. Logistic regression analysis showed that the EDV, PSV, FSH, testosterone level and bilateral testicular volume (BTV) were significant predictors of improvement.

Conclusion: Positivity for ASAs is not a predictor of the outcome after varicocelectomy but affects only the motile fraction in positive cases, despite the improvement in other seminal variables and testicular haemodynamics, and regardless of the surgical approach. The EDV, PSV, FSH, testosterone and BTV were significant predictors of a successful outcome.

Introduction

A varicocele is the pathological dilatation of spermatic veins and is found in ≈15% of all adult males [1], in 11.7% of men with a normal semen analysis and in 25.4% of men with abnormal semen values [2], and it is considered to be the most frequent correctable cause in 14.8% of infertile men [3]. Surgical ligation of the spermatic vein is the generally accepted treatment, when semen quality usually improves afterwards, as shown in a recent meta-analysis [4], and with reversal of any DNA damage [5]. Varicocele has been associated with testicular dysfunction through several mechanisms, such as the retrograde flow of toxic metabolites from the adrenal glands or kidney, venous stasis with germinal epithelial hypoxia, alterations in the hypothalamic-pituitary-gonadal axis, and increases in testicular temperature [6]. Anti-sperm antibodies (ASAs) are an important cause of infertility, found in 8–21% of infertile men [7] and adversely affecting fertility in patients with varicocele, with a small but significant decrease in both sperm concentration and motility [8]. Sperm-bound immunoglobulins are present in a greater percentage of infertile men with varicocele than in infertile men with no varicocele [9]. The testis needs a good blood supply to maintain its function. As postulated in experimental studies [10,11], with varicocele-impaired venous drainage and an increased venous pressure, the blood supply and microperfusion of the testes can be decreased by down-regulating arterial inflow to maintain the homeostasis of the intratesticular vascular pressure. The peak systolic velocity (PSV) and resistive index (RI) were found by some authors [12] to be higher in patients with varicocele (fertile or not) than in fertile control men, and an increased RI might be suggestive of a pathological sperm count [13]. Recent opinion suggests that varicocele is a cofactor associated with other genetic and molecular factors resulting in infertility [14]. In previous studies investigating the predictors of improvement after varicocelectomy, some authors [15] highlighted the role of ASAs while others [12,13] investigated the role of intratesticular haemodynamics. Hormonal levels, testicular volume and varicocele grade were also investigated [16] as predictors of improvement. With these perspectives, the aim of the present study was to assess the probable role of the combined variables of ASAs, hormonal levels, ultrasonographically estimated testicular volume and intratesticular haemodynamics on the outcome of varicocelectomy in infertile men, as expressed by improvements in seminal variables. In addition, the intratesticular haemodynamic changes in infertile patients with varicocele were assessed and compared according to the surgical approach of varicocelectomy.

Patients and methods

Between August 2011 and May 2013, 82 infertile men with abnormal seminal values associated with a clinical varicocele were referred for varicocelectomy and evaluated in a prospective, open-label observational study. Patients with documented primary (60) or secondary (22) infertility for >1 year, with unilateral or bilateral varicocele and abnormal seminal variables were included in the study. Patients with azoospermia and marked oligozoospermia (sperm count $<$ 5 million/mL), recurrent varicocele, cryptorchidism, a history of exposure to gonadotoxins, or known genetic abnormalities and infectious inflammatory processes of the ancillary genital glands (leucocyte count $>$ 1 million/mL) were excluded. Those with a history of testicular trauma, epididymitis, orchitis or a previous surgical intervention of the scrotum were also excluded. The study was approved by the ethics committee of the Benha Faculty of Medicine. Informed consent for participation was obtained from all patients, and the procedure and possible risks explained thoroughly, according to Declaration of Helsinki. In all, 82 infertile patients (median age 31 years, range 20–46) were treated with varicocelectomy; 47 had bilateral and 35 a left varicocele, and abnormal seminal values below the accepted lower limits according to the WHO parameters [17] in at least two semen analyses. Specimens were obtained by masturbation after 2–7 days of sexual abstinence, $\leqslant$4 weeks before surgery, and during the follow-up period the semen analyses were repeated starting $\geqslant$3 months after surgery, comparing the seminal variables of count, motility and percentage of abnormal forms, and the best value adopted. The total motile sperm count (TMSC) was calculated as (ejaculate volume × concentration × motile fraction). The improvement was considered significant when there was a $\geqslant$50% increase from the baseline level of TMSC [18], and as 'unimproved' if not. Before surgery all patients had levels below the accepted lower reference limits, these being; volume 1.5 mL, concentration 15×10^6/mL, total count 39×10^6 per ejaculate, motility 40% (progressive and nonprogressive), and normal forms 35% [17].

The direct SpermMAR IgG Test (FertiPro N.V., Beerem, Belgium) was used as described previously [19]. In this assay the diagnosis of immunological infertility is suspected when 10–39% of the motile spermatozoa are covered by latex particles; if $\geqslant$40% of the spermatozoa are covered, immunological infertility is highly probable.

The patients were examined by grey-scale and duplex colour Doppler ultrasonography (CDUS) using 7.5 MHz probe, during normal respiration and during a Valsalva manoeuvre (Samsung-Medison, Model Accuvix A30, Samsung Co. LTD, Daeschi-Dong, Korea). Scrotal CDUS was undertaken in a warm room with the patient supine, and the penis resting on the lower abdomen. The testicular volume was calculated automatically by the integral software, using the ellipsoid formula, after measuring the three longest diameters with electronic callipers. For diagnosing varicocele, the criteria adopted were; > 2 mm diameter of a vein of the pampiniform plexus, and a reverse flow duration of > 1 s on a Valsalva manoeuvre with an increase in vein diameter of > 3 mm [20]. The varicocele of the pampiniform plexus was graded as follows: grade 0 (no dilated vein), grade 1 (dilated veins < 2.5 mm in diameter with no flow reversal after Valsalva manoeuvre), grade 2 (dilated and tortuous veins 2.5–3.5 mm in diameter and flow reversal after a Valsalva manoeuvre), and grade 3 (dilated and tortuous veins > 3.5 mm in diameter and flow reversal after a Valsalva manoeuvre) [21]. All ultrasonographic studies were performed by one experienced examiner (A.M.A.), unaware of the findings of the physical examination, to prevent bias. CDUS measurements were recorded using an average of three consecutive measurements on tracings including at least five identical waveforms. With power CDUS of the pampiniform plexus, the varicocele was confirmed by measuring the PSV, EDV, RI, and pulsatility index (PI) of the intratesticular branches of the testicular artery of each testicle, comparing the right and left sides in all cases to detect haemodynamic changes in relation to the presence of varicocele. Serum FSH and luteinising hormone (LH) were measured using chemi-luminescence assays, and testosterone was analysed by radioimmunoassay.

The patients were randomly allocated to have either a testicular artery- and lymphatic-sparing inguinal varicocelectomy using ×3 loupe magnification (42), or a high-ligation (Palomo) technique, with preservation of the artery if possible (40). As a primary outcome measure, semen analyses were repeated starting $\geqslant$3 months after surgery (adopting the best reading) and compared to values before surgery. In addition, the intratesticular haemodynamics were reassessed and measurements were recorded for comparison with the preoperative findings. Patients were instructed to avoid using any medications that might affect their semen quality or fertility potential throughout the baseline and study periods.

Results are expressed as the mean (SD, range). Student's *t*-test for paired and unpaired samples was used to compare continuous data between the groups, as appropriate. Spearman's rho and the Pearson correlation coefficient were used to assess any correlation between the postoperative TMSC and nonparametric and continuous data. Binary logistic regression analysis was used to identify significant predictors that could affect the outcome of varicocelectomy.

Results

During a median (range) follow-up of 5 (3–12) months, there was a significant improvement in the sperm concentration, percentage motility and TMSC, and a decrease in the percentage of abnormal forms. There was a significant improvement ($\geqslant$50% increase in TMSC) in 52 (63%) patients (Table 1). After surgery the seminal values in ASA-positive cases showed that the motile fraction was significantly lower.

Comparing the haemodynamic values between the right and left sides, there were no significant differences in PSV, EDV, RI and PI, and thus the mean values were calculated and used for the statistical analysis (Table 2). Before surgery there was no significant difference between right and left sides in patients with a left varicocele except for the EDV of the left side, that was lower on the ipsilateral side of the varicocele. After surgery on the right side there was no change in the haemodynamic variables, while the left side showed a significant increase in EDV and decrease in the RI and PI. The haemodynamic values improved on the operated side only, with no significant change on the right side. In bilateral varicocele, there was no difference between the right and left sides before or after surgery, but with a significant increase in the EDV and decrease in RI and PI bilaterally

Table 1 A comparison between seminal values before and after surgery in 82 patients, and those positive or negative for ASAs.

Variable	Before	After	P^a	Preop ASAs		P^b
				Positive	Negative	
N (%)	–	–	–	17 (21)	65 (79)	
Semen volume (mL)	2.57 (0.9)	2.65 (0.75)	0.297	2.38 (0.7)	2.7 (0.8)	0.088
Sperm density (million/mL)	10.52 (2.5)	22.2 (14.6)	< 0.001	18.8 (14)	23.1 (14.7)	0.284
% Motility	43.6 (13.3)	59.3 (11.6)	< 0.001	52.5 (9)	61.1 (11.6)	0.006
Total motile sperm count	10.9 (4.83)	37.1 (28.2)	< 0.001	25.3 (22.9)	40.2 (28.8)	0.052
% Abnormal forms	41.2 (9.2)	27.9 (8.9)	< 0.001	27.2 (7.9)	28.1 (9.3)	0.738

Student's *t*-test for.
[a] Paired samples.
[b] Unpaired samples.

Table 2 A comparison of the mean (SD, range) of intratesticular haemodynamic values in the right and left testes, before and after surgery in patients with left and bilateral varicocele.

Side	PSV (cm/s)	EDV (cm/s)	RI	PI
Left varicocele (35)				
Right				
Before	10.29 (3.06)	3.58 (1.4)	0.65 (0.06)	1.13 (0.15)
P	–	–	–	–
After	9.46 (1.9)	3.52 (1.1)	0.62 (0.09)	1.06 (0.18)
P	–	–	–	–
P	0.177[b]	0.843[b]	0.051[b]	0.088[b]
Left				
Before	9.18 (2.97)	2.74 (1.7)	0.69 (0.13)	1.14 (0.3)
P	0.083[a]	0.010[a]	0.100[a]	0.815[a]
After	8.79 (1.6)	4.49 (1.01)	0.49 (0.05)	0.72 (0.12)
P	0.488[a]	< 0.001[a]	< 0.001[a]	< 0.001[a]
P	0.062[b]	< 0.001[b]	< 0.001[b]	< 0.001[b]
Bilateral (47)				
Right				
Before	10.48 (2.7)	3.81 (1.6)	0.64 (0.11)	1.08 (0.26)
P	–	–	–	–
After	9.72 (2.1)	4.98 (1.3)	0.49 (0.07	0.72 (0.17)
P	–	–	–	–
P	0.136[b]	0.001[b]	< 0.001[b]	< 0.001 [b]
Left				
Before	10.21 (2.7)	3.58 (1.9)	0.66 (0.14)	1.01 (0.25)
P	0.543[a]	0.437[a]	0.395[a]	0.127[a]
After	9.89 (1.8)	5.12 (0.98)	0.47 (0.07)	0.67 (0.12)
P	0.659[a]	0.054[a]	0.546[a]	0.089[a]
P	0.494[b]	< 0.001[b]	< 0.001[b]	< 0.001[b]
Mean (SD, range)				
Before (82)	9.94 (2.3, 5.8–16.7)	3.29 (1.45, 0.79–7.04)	0.65 (0.09, 0.48–0.88)	1.07 (0.19, 0.69–1.58)
After (82)	9.45 (1.48, 6.0–14.5)	4.53 (0.99, 2.6–7.3)	0.50 (0.07, 0.35–0.67)	0.76 (0.13, 0.45–1.06)
P	0.062[b]	< 0.001[b]	< 0.001[b]	< 0.001[b]

Student's *t*-test for paired samples.
[a] Right vs. left.
[b] Before vs. after.

after varicocelectomy. The PSV did not change significantly. In all 82 patients there was a significant increase in the mean values of EDV and a significant decrease in RI and PI, whilst the change in the PSV was insignificant.

There was no significant difference in the postoperative seminal values after unilateral left varicocelectomy vs. bilateral varicocelectomy (Table 3). Also, the outcome after high ligation vs. inguinal was not significantly different. For the haemodynamic values the outcome was significantly in favour of the bilateral varicocelectomy and the inguinal approach. The latter significantly improved the haemodynamic values more than high ligation (Palomo). Of patients considered

Table 3 The outcome after unilateral left vs. bilateral varicocelectomy and high ligation vs. the inguinal approach.

Mean (SD, range) variable	Left varicocele	Bilateral	*P*	High ligation	Inguinal	*P*
N (%)	35 (43)	47 (57)	40 (49)	42 (51)		
Semen volume (mL)	2.69 (0.8, 1.5–4.5)	2.63 (0.8, 1.5–4.5)	0.735[a]	2.5 (0.7, 1.5–4.5)	2.8 (0.7, 1.5–4.5)	0.069[a]
Sperm density (10^6/mL)	20.7 (11.8, 2.5–48.9)	23.3 (16.4, 2.3–62.5)	0.419[a]	20.7 (12.5, 2.3–54.6)	23.6 (16.3, 2.5–62.5)	0.374[a]
% Motility	58.3 (11.2, 45–85)	60 (11.9, 40–85)	0.526[a]	60.3 (9.7, 40–85)	58.3 (13.2, 40–85)	0.436[a]
TMSC	34.0 (24.2, 4.38–79.6)	39.4 (30.9, 2.08–123)	0.397[a]	34.6 (27.7, 2.08–123)	39.5 (28.8, 4.38–110)	0.442[a]
% Abnormal forms	29.7 (12.1,15–55)	26.5 (5.3, 10–40)	0.113[a]	28.6 (7.4, 15–55)	27.2 (10.3, 10–55)	0.473[a]
BTV, mL	30.5 (2.5, 24.9–37.9)	30.7 (3.9, 22.7–38)	0.814[a]	30.9 (3.3, 22.7–38)	30.3 (3.4, 22.8–37.9)	0.375[a]
PSV (cm/s)	9.14 (1.4, 5.99–12.3)	9.67 (1.5, 6–14.5)	0.110[a]	9.09 (1.4, 5.99–11.8)	9.79 (1.5, 6–14.5)	0.031[a]
EDV (cm/s)	3.91 (0.82, 2.63–5.87)	4.99 (0.86, 3–7.32)	< 0.001[a]	4.29 (0.9, 2.63—6.09)	4.77 (1.07, 3–7.32)	0.030[a]
RI	0.55 (0.05,0.46–0.67)	0.47 (0.05, 0.35–0.56)	< 0.001[a]	0.51 (0.06, 0.41–0.67)	0.50 (0.07, 0.35–0.61)	0.277[a]
PI	0.85 (0.12, 0.69–1.06)	0.69 (0.09, 0.45–0.87)	< 0.001[a]	0.79 (0.12, 0. 56–1.06)	0.74 (0.13, 0.45–0.96)	0.067[a]
Improvement, n (%)	21 (60)	31 (66)	0.646[b]	23 (58)	29 (69)	0.360[b]
Hydrocele, *n* (%)	–	–	–	2 (5)	0	0.235[b]
Venous backflow, *n* (%)	–	–	–	7 (18)	1 (2)	0.027[b]

[a] Student's *t*-test for unpaired data, left vs. bilateral varicocelectomy and high inguinal (Palomo) vs. inguinal approach.
[b] Fisher's exact test.

'improved' (as assessed by seminal values), 23/40 (58%) had a Palomo procedure and 29/42 (69%) an inguinal approach, but the difference was statistically insignificant ($P = 0.360$, Fisher's exact test). In addition, comparing those who had a high ligation (Palomo) or inguinal approach, there was a significant difference in venous backflow ($P = 0.027$) that was greater after the Palomo operation, and a hydrocele was detected after the Palomo in two men, although this was statistically insignificant.

Table 4 shows the seminal variables after surgery in the improved and unimproved patients; there was a significant difference in the sperm concentration, motility and TMSC. There was a significant difference between the improved and unimproved patients before surgery, with lower FSH and higher testosterone levels in the improved patients. In addition, the EDV was higher, and RI and PI were significantly lower. For ASAs, eight of 52 (15%) improved men were positive, while nine of 30 (30%) unimproved patients were positive (no significant difference). There were no significant differences in semen values between the groups differing in laterality of varicocelectomy and type of surgical approach, although there was a higher percentage of improvement after inguinal varicocelectomy. Correlation between the postoperative TMSC and the different preoperative variables showed a significant negative correlation with ASA positivity (Spearman's rho = −0.232; $P = 0.036$), while

Table 4 The univariate analysis of improved and unimproved patients.

Variable	Improved	Unimproved	*P*
N (%)	52 (65)	30 (35)	
Semen volume (mL)	2.8 (0.7)	2.5 (0.8)	0.063[a]
Sperm density (million/mL)	29.7 (12.9)	8.99 (4.1)	< 0.001[a]
Motility percentage	64.1 (10.7)	48.6 (8.4)	< 0.001[a]
TMSC	54.5 (24.2)	10.5 (5.2)	< 0.001[a]
Abnormal forms (%)	24.6 (6.5)	33.6 (9.8)	< 0.001[a]
FSH (mIU/mL)	4.79 (1.8)	7.28 (2.75)	< 0.001[a]
Testosterone (mmol/L)	15.56 (3.67)	12.17 (2.5)	< 0.001[a]
LH (mIU/mL)	6.13 (1.4)	6.05 (1.5)	0.800[a]
BTV (mL)	30.9 (3.2)	29.1 (2.9)	0.013[a]
PSV (cm/s)	9.73 (2.08)	10.3 (2.7)	0.285[a]
EDV(cm/s)	3.6 (1.4)	2.77 (1.5)	0.012[a]
RI	0.62 (0.08)	0.72 (0.07)	< 0.001[a]
PI	1.01 (0.18)	1.17 (0.16)	< 0.001[a]
ASA positivity, n (%)	8 (15)	9 (30)	0.158[b]
Left varicocele (35), *n* (%)	21 (60)	14 (40)	
Bilateral varicocele (47), *n* (%)	31 (66)	16 (34)	0.646[b]
High ligation (40), *n* (%)	23 (57.5)	17 (42.5)	
Inguinal approach (42), *n* (%)	29 (69)	13 (31)	0.360[b]

[a] Student's *t*-test for unpaired samples.
[b] Fisher's exact test.

a correlation with the summed grade of varicocele and bilaterality was not significant (Spearman's $r = -0.022$ and 0.043; $P = 0.845$ and 0.700, respectively). The correlation with preoperative FSH and testosterone level was significantly negative for FSH and positive for testosterone ($r = -0.433$ and 0.337, and $P < 0.001$ and 0.002, respectively). There was a significant positive correlation with preoperative EDV ($r = 0.326$ and $P = 0.003$), and a negative correlation with RI and PI ($r = -0.453$ and -0.360; both $P < 0.001$, respectively). The correlation with bilateral testicular volume (BTV) was significantly positive ($r = 0.293$; $P = 0.007$), whilst the correlation with PSV was not significant.

To identify the preoperative variables that could affect the outcome after varicocelectomy, FSH and testosterone level, ASA positivity, sum of varicocele grade, laterality of the varicocelectomy, BTV, mean PSV and mean EDV were entered into a binary logistic regression analysis. Table 5 shows the coefficient of regression, Wald test, and the odds ratio with 95% CI from the analysis, where these factors were suggested to affect the outcome of varicocelectomy and the improvement after surgery. The analysis showed that preoperative FSH and testosterone levels, PSV, EDV and preoperative BTV were significant predictors of improvement. The FSH level and mean preoperative PSV had a negative effect on the improvement. From Table 5, by inverting the odds ratio of PSV and FSH, the patient would not improve by 3.06 and 1.47 times for each unit increase in PSV and FSH. From the odds ratio of EDV and testosterone, in the opposite direction, a one point increase in EDV and testosterone is associated with the odds of improvement increasing by 5.2 for EDV and 1.3 for testosterone.

Discussion

The effect of a varicocele on fertility is documented in many studies as causing semen abnormalities, a decreased testicular volume, and a decline in Leydig cell function [22]. A recent meta-analysis suggested that varicocelectomy leads to significant improvements in the sperm count and motility regardless of surgical technique, with the inguinal approach offering the highest paternity rate [23,24]. The present study was conducted to assess the probable factors that could affect the outcome of varicocelectomy, i.e., the role of positivity for ASAs, intratesticular haemodynamic values, and hormonal levels in infertile men with varicocele and abnormal seminal values. In addition, we compared the intratesticular haemodynamic changes before and after varicocelectomy according to the surgical approach.

The prevalence of ASAs in the general population is 0–2%, and is up to 28% in patients with varicocele [25,26]. ASAs are thought to result from a disruption of the blood–testis barrier (BTB), but in case of varicocele the mechanism of the production of ASAs is unclear. Turner et al. [27] found that the BTB was not damaged in experimentally induced unilateral left varicocele, with a bilateral change in testicular and epididymal function that is not caused by altering the BTB, epididymal temperature or electrolyte concentrations, or testicular blood-gas concentrations. In the present study, the prevalence of ASAs was 21%, whilst the prevalence in the previously cited studies was 26% [25] and 28% in 32 infertile patients with varicocele [26]. The positivity of ASAs before surgery did not preclude the improvement in seminal values after surgery. Although the postoperative motile fraction was significantly lower in ASA-positive men, the sperm count, TMSC and percentage of abnormal forms were unaffected by the presence of ASAs before surgery. This finding is in agreement with many studies [25,28], whilst others [15] found that in men with varicocele the autoimmune antisperm reaction was accompanied by a more significant decrease in the semen quality (count and number of progressively motile and morphologically normal spermatozoa in the ejaculate).

Testicular function and spermatogenesis require a good blood supply, and several studies [12,13] investigated the effect of a varicocele on testicular blood flow as it could be a reliable indicator for spermatogenesis. Doppler indices have been used to obtain information about blood flow and vascular impedance that cannot be obtained from velocity information alone. These indices depend on the measurements of PSV, EDV, RI and PI [13]. There was no control group in the present study, so we compared the blood flow of the right and left

Table 5 The coefficient of regression, Wald test, and odds ratio (95% CI) on the logistic regression analysis.

Variables	B	Wald	P	Odds ratio (95% CI)
Mean pre-op PSV	−1.119	8.536	0.003	0.327 (0.154–0.692)
Mean pre-op EDV	1.652	7.783	0.005	5.215 (1.634–16.64)
FSH	−0.385	4.584	0.032	0.680 (0.478–0.968)
Testosterone	0.278	4.958	0.026	1.321 (1.034–1.687)
ASAs (1)	−1.168	1.408	0.235	3.214 (0.467–22.12)
Operation (1)	1.531	3.070	0.080	4.624 (0.834–25.64)
Sum of varicocele grade	0.335	0.753	0.385	1.397 (0.656–2.975)
Pre-op BTV	0.444	6.404	0.011	1.558 (1.105–2.197)
Constant	−10.196	3.085	0.079	0

testes in unilateral and bilateral cases, to investigate any differences between the sides. We consider that measurements within the testicular parenchyma might be more reliable, as many investigators [29,30] have reported the presence of several arterial branches within the inguinal spermatic cord, and thus measuring blood flow in this region might not be sufficient to assess changes in testicular microcirculation. Table 2 shows the comparison of intratesticular haemodynamic variables in the right and left testes before and after surgery in patients with left or bilateral varicocele. Before surgery there was no significant difference between the right and left sides in men with a left varicocele, except for the EDV that was lower on the ipsilateral side of the varicocele. After surgery, in the right side there was no change in the haemodynamic values, whilst the left side had a significant increase in EDV and decrease in the RI and PI. The haemodynamic values improved on the operated side only, with no significant change on the right side. Pinggera et al. [13] found a mean (SD) RI of 0.68 (0.06) in 80 patients with pathological sperm counts, and of 0.54 (0.05) in 80 men with normal seminal values. In another study of 15 patients with a left varicocele and 34 normal controls, the authors [31] found an RI of 0.68 (0.04) and PI of 1.22 (0.15) in the patients. In the intratesticular branches, the RI of the control group was 0.58 (0.05) and 0.57 (0.06) on the right and left sides, respectively. These findings are comparable with the results of the present study.

The present results are in agreement with another study by Balci et al. [18], of 26 infertile men assessed at 6 months after varicocelectomy, when there was a statistically significant improvement in seminal values and an increased mean (SD) EDV, from 3.33 (0.88) to 3.91 (0.84) cm/s, with a reduced RI from 0.585 (0.068) to 0.493 (0.055) and PI from 0.94 (0.2) to 0.71 (0.130 after surgery, whilst the PSV of 8.13 (2.28) reduced insignificantly to 7.8 (1.81). The improved testicular blood flow after varicocelectomy was also confirmed in a recent study by Tarhan et al. [32], who found improved testicular blood perfusion after left varicocelectomy in 30 patients with a clinical varicocele, with no change in the haemodynamic values on the right side. The findings were similar in the present study; in men with bilateral varicocele there was no difference between the right and left sides before and after surgery, but with a significant increase in the EDV and decrease in RI and PI bilaterally after varicocelectomy, and the PSV did not change significantly. In all 82 patients there was a significant increase in EDV and significant decrease in RI and PI, whilst the change in the PSV was insignificant. These data support the view that testicular microcirculation increases after varicocelectomy. Decreases in resistance indices are indicators of an increase in blood flow in the testes after surgery [32]. The seminal values improved after varicocelectomy, confirming the relationship between improved sperm quality and increased testicular blood flow. Despite the insignificant difference between men treated by high ligation or an inguinal approach in the TMSC after surgery, the haemodynamic outcome was significantly in favour of the bilateral varicocelectomy and the inguinal approach, which significantly improved the haemodynamic values more than high ligation (Table 3), possibly because of the precise control of venous ligation in this approach. Persistent back flow after varicocelectomy was found in eight cases, i.e., seven (18%) after high ligation and one (2%) after inguinal varicocelectomy (a significant difference, $P = 0.027$, Fisher's exact test). A hydrocele formed in two (5%) cases after the Palomo technique, with reported overall hydrocele formation rates of 8.2% after that technique. The rates of varicocele recurrence and hydrocele formation steadily decreased with increasing magnification [24].

Comparing the improved and unimproved cases (Table 4) for seminal values, the overall improvement of 63% is comparable with value in many recent studies [33,34]. A lower FSH and higher testosterone levels characterised improved patients, and can be explained by the deficient secretory function of Leydig cells that has been reported in animals with varicocele [27]. The testicular function of patients with a high serum FSH and low testosterone level might be strongly affected by varicocele [16]. In the present study, the correlation of postoperative TMSC with preoperative FSH and testosterone levels was significantly negative, which is in agreement with a previous study [16]. In addition, the EDV was higher and RI significantly lower in improved patients, indicating good testicular microperfusion and spermatogenesis [12,13,32]. The number of men positive for ASAs was significantly higher in the unimproved cases, as ASAs affect the motility of the sperms [7,9,15,28]. The laterality of the varicocelectomy and type of operation were not significantly different between the subgroups. Seminal values improved regardless the technique [23]. EDV, RI, and PI differed significantly between the improved and unimproved cases, with a higher EDV and lower RI and PI, in agreement with many previous studies [16]. There was also a significant positive correlation with preoperative EDV and negative correlation with RI and PI. The correlation with BTV and PSV was not significant.

For predicting the outcome after varicocelectomy, some studies reported that an improvement in semen quality was positively related to varicocele size. Steckel et al. [35] reported that there was a significant improvement in men with a high grade of varicocele after microsurgical ligation, while others [36] stated that a larger varicocele caused irreversible testicular damage and patients showed less improvement. In the present study, the correlation between the postoperative TMSC and grade of varicocele was statistically insignificant. Kondo et al. [16] investigated the predictors of an improvement in semen characteristics after low ligation in 97 oligozoospermic patients with varicocele, with improved seminal values in 55 (57%). In a logistic regression analysis, they found that preoperative serum FSH and testosterone concentrations were predictors of an improvement in sperm concentration. A low serum FSH and high testosterone level are significant factors for predicting an improvement in semen characteristics before low ligation. In the present study, a univariate analysis (Table 4) indicated that improved cases had significantly lower preoperative FSH and higher testosterone levels. There was also a significantly higher preoperative EDV than in the unimproved cases. These results confirm that patients with preoperative low FSH and high testosterone levels associated with good testicular blood perfusion are more likely to improve after varicocelectomy. The improved patients were evaluated retrospectively for preoperative data that could affect the outcome, using binary logistic regression analysis (multivariate analysis) to identify preoperative predictors of the improvement in seminal values. The variables used in the regression analysis were those assumed to affect the outcome, and using $P < 0.05$ to indicate statistical significance, the FSH and testosterone levels, mean preoperative PSV, EDV and preoperative BTV had significant partial effects on improvement, as significant predictors. Testosterone level, EDV and BTV had a positive effect but FSH and PSV had a negative effect on the improvement.

However, more patients need to be assessed to confirm these findings, as a limitation of the present study was the relatively few patients included and the short follow-up period. The paternity rates were not assessed, as this was beyond the scope of the present study.

In conclusion, from the present results, although positivity for ASAs affected the motile fraction, this should not be considered as an obstacle to abandon intervention, as it will not hinder the improvement after varicocelectomy in seminal and haemodynamic values. However, a long-term follow-up and controlled studies are needed, along with other preoperative treatment protocols, in patients positive for ASAs. Infertile patients with varicocele are more likely to improve when they have a high EDV, high testosterone level, low FSH levels, and normal BTV before surgery, suggesting that those with good testicular microperfusion and normal Sertoli-cell and normal secretory function of Leydig cells benefit from repair regardless of the surgical approach in this group of patients. The intratesticular haemodynamics should be described in reports of ultrasonographic examinations for scrotal varicocele, and can be used in the follow-up of patients with varicocele to obtain an accurate estimate of the recurrence and success rates after varicocelectomy.

Conflict of interest

None declared.

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None.

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