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ORIGINAL ARTICLE

Male Health

Intense venous reflux, quantified by a new software to analyze presurgical ultrasound, is associated with unfavorable outcomes of microsurgical varicocelectomy

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The hemodynamic characteristics of venous reflux are associated with infertility in patients with varicocele; however, an effective method for quantifying the structural distribution of the reflux is lacking. This study aimed to predict surgical outcomes using a new software for venous reflux quantification. This was a retrospective cohort study of a consecutive series of 105 patients (age range: 22–44 years) between July 2017 and September 2019. Venous reflux of the varicocele was obtained using the Valsalva maneuver during scrotal Doppler ultrasonography before microsurgical varicocelectomy. Using this software, the colored reflux signals were segmented, and the gray scale of the color pixels representing the reflux velocity was comprehensively quantified into the mean reflux velocity of the green layer (MRVG) and the reflux velocity standard deviation of the green layer (RVSDG). Spontaneous pregnancy and changes from baseline in the semen parameters were assessed during a 12-month follow-up period. Data were analyzed using logistic regression analysis. An association of the high MRVG group with impaired progressive motility (odds ratio [OR] = 2.868, 95% confidence interval [CI]: 1.133–7.265) and impaired sperm concentration (OR = 2.943, 95% CI: 1.196–7.239) was found during multivariate analysis. High MRVG (OR = 2.680, 95% CI: 1.086–6.614) and high RVSDG (OR = 2.508, 95% CI: 1.030–6.111) were found to be independent predictors of failure to achieve pregnancy following microsurgical repair. In summary, intense venous reflux is an independent predictor of impaired progressive motility, sperm concentration, and pregnancy outcomes after microsurgical varicocelectomy.

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INTRODUCTION

Internal spermatic vein (ISV) reflux, which contains metabolic products, is considered to be toxic for testicular function.[1] The complex structure of the pampiniform plexus causes notable diversity of venous hemodynamics,[2] which might have a different impact on spermatogenesis. Varicocelectomy is a commonly used treatment modality that aims to improve semen quality and pregnancy outcomes in infertile men with varicoceles and affected semen parameters.[3] However, there has been a great deal of controversy about the role of varicocele repair in male infertility.[4–6] Since varicocelectomy is a procedure that counters ISV reflux,[7,8] it is reasonable to hypothesize that subgrouping these patients according to the distribution characteristics of the venous reflux may optimize their outcomes. Unfortunately, existing methods for quantifying the structural distribution of ISV reflux are unsatisfactory. Although venography remains the gold standard for ISV reflux diagnosis, it can provide comprehensive information on the structural distribution of ISV.[9,10] Injection of a contrast may change the flow volume and pressure in the ISV system, which makes it difficult to show the natural status of reflux hemodynamics.[11] In this study, we aimed to test our hypotheses by developing new analytical software that analyzed scrotal ultrasonography to investigate the relationship between the distribution characteristics of ISV reflux and the outcomes following microsurgical varicocelectomy.

PATIENTS AND METHODS

Study design and settings

In this retrospective cohort study, 132 consecutive patients who underwent microsurgical varicocelectomy at Shengjing Hospital of China Medical University (Shenyang, China) between July 2017 and September 2019 were reviewed. The patients were evaluated in the Andrology Clinic of the Department of Urology of Shengjing Hospital of China Medical University to reduce technical bias.

Presurgical examination

Within a 3-month presurgical period, all the patients were referred to the same laboratory at our institution twice after 2–5 days of sexual abstinence for semen analysis, including semen volume, pH, progressive

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motility, sperm concentration, and morphology. The levels of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (T), and prolactin (PRL) were measured in each patient. Scrotal ultrasonography with Doppler color flow was performed before microsurgical varicocelectomy at the Department of Ultrasound, Shengjing Hospital of China Medical University. We used Toshiba Aplio 400 (Toshiba Medical System [China] Co., Ltd., Beijing, China), Toshiba Aplio 500 (Toshiba Medical System [China] Co., Ltd.), and a relevant high-frequency (6–12 MHz) linear probe (Toshiba Medical System [China] Co., Ltd.). The Valsalva maneuver was performed to create spermatic venous reflux, and the transverse plane was displayed to detect the distribution of the reflux signals. The color box was adjusted to cover all the ISVs on the screen. The velocity scale and color gain were manipulated until color noise first became apparent in the image background.[12] The Valsalva maneuver was performed three times, images with maximal reflux were obtained, and the maximum venous diameter was measured.[13] A maximal reflux was defined as a flow with the largest area of Doppler signal during the last 3 s before exhalation. Scrotal ultrasonography was performed by PW (a sonographer with 12 years of diagnostic experience in andrological ultrasonography).

Surgical procedure

Subsequently, a subinguinal microsurgical varicocelectomy with arterial and lymphatic sparing was performed. The external spermatic veins were then ligated. Surgical repair of the varicocele was performed by XWX (urologist with 10 years of experience in andrology).

Outcome measures

The primary outcome measure was spontaneous pregnancy, and the secondary outcomes were changes from the mean baseline of each semen parameter. All patients were contacted via telephone. The patients were followed for 12 months after varicocelectomy, and any spontaneous pregnancy was documented. Seminal analyses were performed at 3 months, 6 months, and 12 months of follow-up. Improved progressive motility was defined as an increase in progressive motility following varicocelectomy. Based on preoperative sperm concentration, the following four conditions were defined as improved sperm concentration: an increase in sperm concentration from $<1 \times 10^6$ ml^{-1} to $>5 \times 10^6$ ml^{-1}; from 1–5 $\times 10^6$ ml^{-1} to $\geq 10 \times 10^6$ ml^{-1}; from 5–15 $\times 10^6$ ml^{-1} to $\geq 15 \times 10^6$ ml^{-1}; or from $\geq 15 \times 10^6$ ml^{-1} to a higher value after surgery. Based on preoperative morphology, the following two conditions were defined as improved morphology: an increase in normal sperm morphology from <4% to ≥4% or from ≥4% to a higher normal sperm morphology value after surgery. Otherwise, the semen quality was considered to be impaired following surgical repair.[14]

Inclusion criteria

Patients considered for inclusion were 20- to 45-year-old married infertile men (unprotected sex for 12 months without conceiving) with a clinically significant varicocele (grades 1–3), pathological semen parameters (with at least one of the following semen characteristics: sperm concentration $<10 \times 10^6$ ml^{-1}, progressive motility <35%, or normal sperm morphology <4%), and those who underwent microsurgical varicocelectomy.[15] Semen samples were analyzed according to the World Health Organization semen manual guidelines.[16] Physical examination was used as the reference standard for varicocele diagnosis and grading,[17] which was based on the modified criteria of Dubin and Amelar as follows: (1) absent (no palpable varicocele), (2) grade 1 (palpable varicocele with aid of the Valsalva maneuver), (3) grade 2 (palpable varicocele without Valsalva), and (4) grade 3 (visible varicocele).[18]

Exclusion criteria

Patients meeting the following criteria were considered ineligible: recurrent varicocele following surgical repair, normal semen parameters, smoking, additional causes of infertility (obstructive azoospermia, complete deletion of azoospermia factor gene [AZF]a, AZFb or AZFc, or diabetes), or female factor infertility (medical history, hormonal levels, and hysterosalpingogram of the female partner were warranted from the outset).[19,20]

Image processing and data collection

The images to be analyzed were digital images created using an ultrasound system and downloaded from a picture archiving and communication system (PACS). Image processing and data collection were performed by two of the coauthors (KY and BBC) according to the protocol using Varicocelaid 1.0 encoded by Python 3.8 (Python Software Foundation, Fredericksburg, VA, USA). The software Varicocelaid 1.0 can be downloaded online (https://pan.baidu.com/s/1ZZ3jT4JBBQgCwU0B85yJcQ?pwd=wqro, last accessed on June 24, 2022).

Because the velocity and direction of blood flow were represented by different colors in the color flow ultrasound, the gray value profile of the velocity scale was plotted, and the velocity scale was calibrated to identify the reflux velocity of each pixel in the image (**Supplementary Figure 1a**). To segment the reflux signals, the reflux signals were selected, and the black and white backgrounds were removed (**Supplementary Figure 1b**). Reflux signals were quantified as the mean reflux velocity of the green layer (MRVG) by measuring the mean gray value representing the velocity of the blood flow, and the reflux velocity standard deviation (s.d.) of the green layer (RVSDG) by measuring the s.d. of the gray value representing the blood flow velocity (**Supplementary Figure 1c**). The results for each parameter were obtained from three independent measurements.

Protocol for reflux signal segmentation and quantification

Single-blinded reflux signal segmentation and quantification were performed by KY and BBC according to the following protocol: (1) enter the maximum velocity scale; (2) upload the image to be detected; (3) indicate four edge points, including the upper left corner of the velocity scale, lower right corner of the velocity scale (chromatography to indicate the velocity and direction of the blood flow in the image), upper left corner of the region of interest (area to be measured with all reflux signals), and lower right corner of the region of interest (**Supplementary Figure 1a**); (4) close the dialog box and read the results, including MRVG and RVSDG; and (5) if there was a difference or disagreement in the results for a specific sample, a repeated single-blinded measurement was performed by another author, and the final result was determined by an author meeting.

Green layer of the red, green, and blue (RGB) image stack and standardized value of the MRVG and RVSDG

To avoid missing data and minimize bias, the green layer of the RGB stack was chosen for analysis. The reason for selecting the green layer of the RGB stack to evaluate the reflux velocity was that the gray value profile of the velocity scale in the green layer was distributed in a linear and symmetric manner so that the measured velocity would not be biased by the blood flow direction (**Supplementary Figure 2a**). A positive correlation was observed between the velocity scale and MRVG (**Supplementary Figure 2b**) and RVSDG (**Supplementary Figure 2c**). The correlation between color gain and MRVG/RVSDG was not statistically significant (data not shown). To minimize the bias caused by the velocity scale and to avoid indeterminate results, the MRVG and RVSDG were divided by the velocity scale, and the standardized values of the MRVG (**Supplementary Figure 2b**) and RVSDG (**Supplementary Figure 2c**) were obtained. Since the processes of image acquisition, modification, and data collection were performed consistently according to the protocols as planned, there were no technical differences that affected the reliability of the study's results.

Statistical analyses

Ward's clustering method with Euclidean distance, as described in Pepin *et al.*,[21] was used to subgroup patients with varicocele into low and high groups. Student's *t*-test was used for comparison of numerical variables; the Chi-square test was used for comparison of rates of categorical variables; binary logistic regression was used for univariate and multivariate analyses; and $P < 0.05$ was considered statistically significant. Statistical analyses were carried out using IBM SPSS Statistics version 21 (IBM, Armonk, NY, USA), including Student's *t*-test, the Chi-square test, and logistic regression, and GraphPad Prism 7 software (GraphPad Software, San Diego, CA, USA) was used to plot the scattered dot graphs.

Ethical statement

The authors are accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (revised in 2013). The study was approved by the Ethics Committee of Shengjing Hospital of China Medical University (ethics approval No. 2021PS512k), and all the participants provided informed consent before their inclusion in the study.

RESULTS

Comparison of the baseline characteristics of the MRVG and RVSDG between the low and high groups

In this study, 105 patients were enrolled (**Figure 1**). The follow-up period (mean ± s.d.) was 37.9 ± 13.4 months. The baseline characteristics of the study population are shown in **Table 1**. We found that higher MRVG signals tended to have brighter colors and that higher RVSDG signals tended to show a more complex color style (**Figure 2**). Intraobserver and interobserver variabilities of MRVG were 4.2% and 9.8%, respectively, while those of RVSDG were 5.4% and 12.7%, respectively. The left maximum venous diameters in the low group of MRVG ($P < 0.01$) and RVSDG ($P < 0.01$) were lower than those in the high group. Other demographic and clinical characteristics were comparable between the subgroups with nonsignificant differences (**Table 2**).

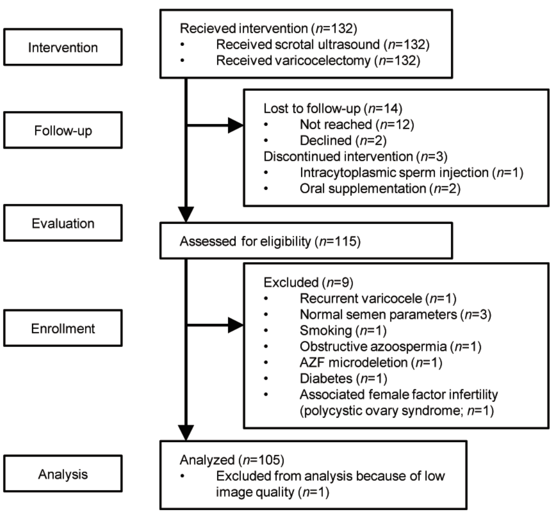

Figure 1: Flow diagram for consolidated standards of the study participants. AZF: azoospermia factor gene.

Table 1: The baseline characteristics of the study population (*n*=105)

Characteristic	*Values*
Age of participants (year), mean±s.d. (range)	30.2±4.8 (22–44)
Age of wives (year), mean±s.d. (range)	28.6±4.4 (20–41)
BMI (kg m^{-2}), mean±s.d. (range)	23.5±4.2 (15.0–35.0)
Left maximum venous diameter (cm), mean±s.d. (range)	0.32±0.07 (0.19––0.60)
Right maximum venous diameter (cm), mean±s.d. (range)	0.19±0.08 (0.07–0.49)
Left testicular volume (ml), mean±s.d. (range)	13.7±4.0 (5.0–27.0)
Right testicular volume (ml), mean±s.d. (range)	14.5±3.9 (7.9–28.0)
Velocity scale (cm s^{-1}), mean±s.d. (range)	4.2±1.7 (1.4–10.0)
Color gain (%), mean±s.d. (range)	50.8±16.8 (20.0–85.0)
Baseline semen parameters, mean±s.d. (range)	
Progressive motility (%)	19.5±14.2 (0.2–77.9)
Sperm concentration (×10^6 ml^{-1})	20.0±22.3 (0.2–150.0)
Normal morphology (%)	3.5±1.9 (0.2–7.0)
Reproductive hormone level, mean±s.d. (range)	
FSH (mIU ml^{-1})	5.4±2.1 (1.7–10.3)
LH (mIU ml^{-1})	4.5±1.5 (2.0–8.8)
T (ng ml^{-1})	3.7±1.2 (1.7–7.4)
PRL (ng ml^{-1})	13.3±5.5 (5.1–48.6)
Infertility duration (month), mean±s.d. (range)	20.9±11.3 (12.0–72.0)
Infertility, *n*/total (%)	
Primary	76/105 (72.4)
Secondary	29/105 (27.6)
Surgical repair side, *n*/total (%)	
Bilateral	16/105 (15.2)
Unilateral (left)	89/105 (84.8)
Varicocele grade, *n*/total (%)	
Grade 1	14/105 (13.3)
Grade 2	37/105 (35.2)
Grade 3	54/105 (51.4)

FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: total testosterone; PRL: prolactin; BMI: body mass index; s.d.: standard deviation

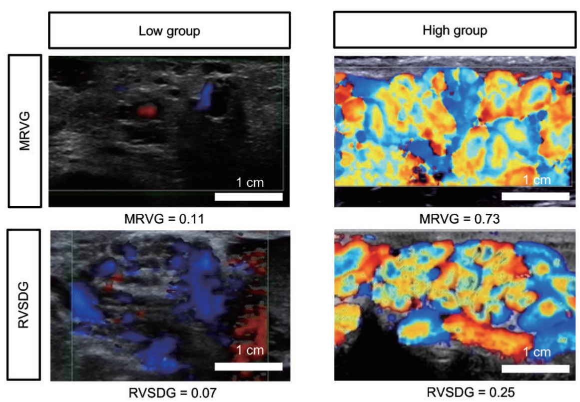

Figure 2: Reflux signal distribution of MRVG and RVSDG in low and high groups (scale bars = 1 cm). MRVG: mean reflux velocity of green layer; RVSDG: reflux velocity standard deviation of green layer.

Table 2: The baseline characteristics of subgroups of mean reflux velocity of green layer and reflux velocity standard deviation of green layer (*n*=105)

Parameter	*MRVG*			*RVSDG*		
	Low group	*High group*	*P*	*Low group*	*High group*	*P*
Participant (*n*)	52	53	-	54	51	-
MRVG/RVSDG, mean±s.d.	0.21±0.05	0.42±0.09	<0.001	0.15±0.03	0.25±0.02	<0.001
MRVG/RVSDG, range	0.11–0.30	0.30–0.73	-	0.07–0.20	0.20–0.30	-
Age of participants (year), mean±s.d.	31.0±4.3	29.5±5.2	0.118	30.9±4.5	29.5±5.1	0.122
Age of wives (year), mean±s.d.	28.9±3.9	28.3±4.9	0.475	29.0±3.9	28.1±4.9	0.309
BMI (kg m^{-2}), mean±s.d.	23.3±4.2	23.7±4.2	0.624	23.5±3.9	23.5±4.4	0.913
Maximum venous diameter (cm), mean±s.d.						
Left	0.30±0.04	0.34±0.09	<0.01	0.30±0.05	0.34±0.09	<0.01
Right	0.19±0.07	0.19±0.08	0.944	0.19±0.07	0.20±0.09	0.347
Left testicular volume (ml), mean±s.d.	14.4±4.4	13.0±3.4	0.072	14.1±4.2	13.3±3.8	0.264
Right testicular volume (ml), mean±s.d.	15.1±4.5	13.8±3.0	0.099	14.5±3.8	14.4±3.9	0.919
Baseline semen parameters, mean±s.d.						
Progressive motility (%)	18.0±12.9	21.0±15.4	0.282	18.8±12.1	20.2±16.3	0.635
Sperm concentration (×10^6 ml^{-1})	23.8±27.0	16.3±15.9	0.086	21.5±24.9	18.5±19.2	0.500
Normal morphology (%)	3.3±1.8	3.6±2.0	0.524	3.2±1.7	3.8±2.0	0.098
Reproductive hormone level, mean±s.d.						
FSH (mIU ml^{-1})	5.0±2.1	5.7±2.1	0.093	5.3±2.2	5.4±2.1	0.945
LH (mIU ml^{-1})	4.5±1.6	4.6±1.5	0.649	4.4±1.6	4.7±1.5	0.381
T (ng ml^{-1})	3.5±1.0	3.9±1.4	0.065	3.6±1.1	3.8±1.4	0.454
PRL (ng ml^{-1})	12.9±4.7	13.8±6.1	0.404	12.7±3.8	14.0±6.8	0.243
Infertility duration (month), mean±s.d.	20.8±9.1	21.0±13.1	0.951	19.8±8.4	22.1±13.7	0.289
Infertility, *n* (%)			0.142			0.087
Primary	41 (78.8)	35 (66.0)		43 (79.6)	33 (64.7)	
Secondary	11 (21.2)	18 (34.0)		11 (20.4)	18 (35.3)	
Surgical repair side, *n* (%)			0.559			0.336
Bilateral	9 (17.3)	7 (13.2)		10 (18.5)	6 (11.8)	
Unilateral (left)	43 (82.7)	46 (86.8)		44 (81.5)	45 (88.2)	
Varicocele grade, *n* (%)			0.282			0.183
Grade 1	9 (17.3)	5 (9.4)		10 (18.5)	4 (7.8)	
Grade 2	15 (28.8)	22 (41.5)		20 (37.0)	17 (33.3)	
Grade 3	28 (53.8)	26 (49.1)		24 (44.4)	30 (58.8)	

MRVG: mean reflux velocity of green layer; RVSDG: reflux velocity standard deviation of green layer; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: total testosterone; PRL: prolactin; s.d.: standard division; -: not available; BMI: body mass index

Poorer outcomes for the high MRVG and RVSDG groups following microsurgical varicocelectomy

The probability of impaired progressive motility (high group: 60.4% *vs* low group: 36.5%) and impaired sperm concentration (high group: 54.7% *vs* low group: 30.8%) was significantly higher in the high MRVG group than in the low group following microsurgical varicocelectomy ($P < 0.05$; **Table 3**). The probability of failing to achieve pregnancy in the high MRVG group (high group: 62.3% *vs* low group: 40.4%) and the high RVSDG (high group: 62.7% *vs* low group: 40.7%) group was significantly higher than that in the low group ($P < 0.05$; **Table 3**).

Association of the high MRVG group with impaired sperm concentration and failure to achieve pregnancy is independent of classical baseline factors

Univariate analyses showed that patients in the high MRVG group were twice as likely to have impaired progressive motility (odds ratio [OR] = 2.647, 95% confidence interval [CI]: 1.203–5.822, $P < 0.05$), impaired sperm concentration (OR = 2.719, 95% CI: 1.222–6.048, $P < 0.05$), and failure to achieve pregnancy (OR = 2.436, 95% CI: 1.112–5.337, $P < 0.05$) as those in the low group following microsurgical varicocelectomy. The high RVSDG group was also associated with failure to achieve pregnancy (OR = 2.450, 95% CI: 1.117–5.373, $P < 0.05$). For predicting impaired progressive motility using MRVG, the area under the receiver operating characteristic curves (AUC) was 0.617 (95% CI: 0.509–0.726, $P < 0.05$; **Supplementary Figure 3a**), positive predictive value (PPV) = 60.4%, and negative predictive value (NPV) = 63.5%. For predicting impaired sperm concentration using MRVG, AUC was 0.646 (95% CI: 0.539–0.753, $P < 0.05$; **Supplementary Figure 3b**), PPV = 54.7%, and NPV = 69.2%. For predicting failure to achieve pregnancy using MRVG, AUC was 0.641 (95% CI: 0.534–0.748, $P < 0.05$; **Supplementary Figure 3c**), PPV = 62.3%, and NPV = 59.6%. For predicting failure to achieve pregnancy using RVSDG, AUC was 0.610 (95% CI: 0.501–0.719, $P < 0.05$; **Supplementary Figure 3c**), PPV = 62.8%, and NPV = 59.3%. Younger age was associated with impaired progressive motility (OR = 1.112, 95% CI: 1.019–1.214, $P < 0.05$). The associations of impaired semen parameters with failure to achieve pregnancy with baseline variables, including body mass index (BMI), left maximum venous diameter, right maximum venous diameter, left testicular volume, right testicular volume, serum FSH, LH, T, PRL, infertility type, infertility duration, surgical repair side varicocele grade, velocity scale, and color gain, were not statistically significant (data not shown).

Multivariate analyses were performed to estimate the predictive value of MRVG and RVSDG for the outcome of microsurgical varicocelectomy. Baseline variables, including age, left maximum venous diameter, BMI, FSH, T, infertility duration, surgical repair side, and varicocele grade, which were considered clinically relevant or showed a univariate relationship ($P < 0.15$) with outcome, were entered into multivariate analyses. The association between high MRVG and impaired progressive motility (OR = 3.521, 95% CI: 1.458–8.501, $P < 0.01$) or impaired sperm concentration (OR = 2.846, 95% CI: 1.214–6.673, $P < 0.05$) was sustained after adjustments for BMI, FSH, T, infertility duration, surgical repair side, and varicocele grade (**Table 4**). Remarkably, the association of the high MRVG group with impaired progressive motility (OR = 2.868, 95% CI: 1.133–7.265, $P < 0.05$) or impaired sperm concentration (OR = 2.943, 95% CI: 1.196–7.239, $P < 0.05$) was sustained even after adjusting for all baseline factors, including age and left maximum venous diameter (**Table 4**). Importantly, the association of the high MRVG group (OR = 2.680, 95% CI: 1.086–6.614, $P < 0.05$) or the RVSDG group (OR = 2.508, 95% CI: 1.030–6.111, $P < 0.05$) with failure to achieve pregnancy was sustained even with adjustments for all of the baseline factors, including age and left maximum venous diameter (**Table 4**).

DISCUSSION

It is widely accepted that the hemodynamics of testicular varicoceles are correlated with spermatogenesis and the effects of varicocelectomy.[22,23] Based on the results of previous studies, which showed that reflux of the ISV contributes to male infertility,[2,24] we developed a new software to quantify the structural distribution of the reflux into two parameters, including MRVG and RVSDG. Furthermore, we observed that high MRVG was an independent predictor of impaired semen quality and that high MRVG and high RVSDG were independent predictors of failure to achieve pregnancy following microsurgical varicocelectomy.

Because the ISV becomes a pampiniform plexus with a complex and diverse structure near the testis[2] and the critical Reynolds number is low enough to cause turbulent flow in branching vessels,[25,26] it is quite challenging to study the hemodynamics of the reflux in this area. This study used a new approach to comprehensively quantify hemodynamic characteristics within this venous system. The color nature of the reflux signal in the Doppler ultrasound image made it distinct from the black and white backgrounds, so that the segmentation and quantification of the reflux signal could be performed precisely. Because the local intensity of green in the color Doppler image represents a statistical measure of the spread of the velocities around each local mean velocity estimate, the green color evaluation of the Doppler ultrasound image was used to improve the detection of circulatory system diseases, such as minimal valve regurgitation jets and renal artery stenosis.[27] Furthermore, superior to red or blue reflux signals, the green color could comprehensively display the velocity level of the venous reflux from all directions (**Supplementary Figure 2a**). Generated by measuring the mean value and its s.d. of the gray value in the green layer of the RGB image, the MRVG and RVSDG represent the average reflux velocity and difference in its structural distribution, respectively. In a previous

Table 3: Comparison of impaired semen parameters and failing to achieve pregnancy between subgroups of mean reflux velocity of green layer and reflux velocity standard deviation of green layer following microscopic varicocelectomy (*n*=105)

Outcome	*Low group, n/total (%)*	*High group, n/total (%)*	*OR (95% CI)*	*P*
MRVG				
Impaired progressive motility	19/52 (36.5)	32/53 (60.4)	2.646 (1.203–5.814)	<0.05
Impaired sperm concentration	16/52 (30.8)	29/53 (54.7)	2.717 (1.222–6.061)	<0.05
Impaired morphology	37/52 (71.2)	31/53 (58.5)	0.571 (0.254–1.285)	0.174
Failing to achieve pregnancy	21/52 (40.4)	33/53 (62.3)	2.433 (1.111–5.348)	<0.05
RVSDG				
Impaired progressive motility	25/54 (46.3)	26/51 (51.0)	1.206 (0.561–2.597)	0.631
Impaired sperm concentration	20/54 (37.0)	25/51 (49.0)	1.634 (0.750–3.559)	0.215
Impaired morphology	35/54 (64.8)	33/51 (64.7)	0.995 (0.447–2.217)	0.991
Failing to achieve pregnancy	22/54 (40.7)	32/51 (62.7)	2.451 (1.117–5.376)	<0.05

The rates were compared using the Chi-square test. MRVG: mean reflux velocity of green layer; RVSDG: reflux velocity standard deviation of green layer; OR: odds ratio; CI: confidence interval

Table 4: Multivariate analyses of the association of mean reflux velocity of green layer and reflux velocity standard deviation of green layer subgroups with the outcomes of microscopic varicocelectomy (*n*=105)

Outcomes	*MRVG high group*		*RVSDG high group*	
	OR (95% CI)	*P*	*OR (95% CI)*	*P*
Adjusted I[a]				
Impaired progressive motility	3.521 (1.458–8.501)	<0.01	1.150 (0.508–2.603)	0.737
Impaired sperm concentration	2.846 (1.214–6.673)	<0.05	1.689 (0.736–3.874)	0.216
Failing to achieve pregnancy	2.696 (1.135–6.402)	<0.05	2.570 (1.087–6.077)	<0.05
Adjusted II[b]				
Impaired progressive motility	2.868 (1.133–7.265)	<0.05	0.853 (0.353–2.062)	0.724
Impaired sperm concentration	2.943 (1.196–7.239)	<0.05	1.636 (0.691–3.875)	0.263
Failing to achieve pregnancy	2.680 (1.086–6.614)	<0.05	2.508 (1.030–6.111)	<0.05

[a]Adjusted for BMI, FSH, T, infertility duration, surgical repair side, and varicocele grade; [b]in addition to adjusted I, also adjusted for left maximum venous diameter and age.
MRVG: mean reflux velocity of green layer; RVSDG: reflux velocity standard deviation of green layer; OR: odds ratio; CI: confidence interval; FSH: follicle-stimulating hormone; T: total testosterone; BMI: body mass index

study, color Doppler ultrasound was used to measure the velocity of spermatic venous reflux;[8] however, the complexity of the ISV vasculature made it difficult to choose a representative vessel for measurement using a gate or sample volume. Because intense reflux intuitively exhibited brighter colors and a complex color style in the ultrasound image, our study quantified these visual characteristics into MRVG and RVSDG.

For the past decade, the duration of ISV reflux has been used to evaluate the reflux intensity of varicocele,[13] while our study provided an option to evaluate the outcome from the perspective of the structural characteristics of this complex venous reflux. Our results demonstrated that higher MRVG was associated with impaired progressive motility following microscopic varicocele repair. We found that the association of high MRVG with impaired progressive motility and impaired sperm concentration persisted after adjusting for BMI, FSH, T, infertility duration, surgical repair side, and varicocele grade. The association of the high MRVG group with impaired progressive motility and impaired sperm concentration was sustained even with adjustment for all of the baseline factors, including age (a candidate variable with statistical significance in univariate analysis) and left maximum venous diameter (a variable with multicollinearity),[28] indicating that high MRVG is an independent predictor of impaired progressive motility and impaired sperm concentration following microsurgical varicocelectomy. High MRVG and RVSDG were also proven to be independent predictors of failure to achieve pregnancy following microsurgical varicocelectomy in multivariate analysis. Our findings indicate that the higher the mean reflux velocity (or the more diverse the reflux velocity distribution), the poorer the outcome. To the best of our knowledge, this study provided a novel method to identify the structural distribution of ISV reflux velocity as an independent predictor of microsurgical varicocelectomy outcome. Persistent reflux after surgical varicocele repair, which is frequently present (18%–48%),[7,29] is considered a risk factor for poor outcomes.[7,8] Another risk factor for the poor outcome of surgical repair is irreversible testicular damage induced by varicocele.[30] Further studies are needed to investigate the potential correlation of high MRVG or RVSDG with postsurgical persistent reflux and varicocele-induced irreversible testicular damage.

Limitations of this study include its retrospective nature. Additionally, varying the intensity of the Valsalva maneuver and moments of maximum reflux may cause bias. This bias could be reduced through standardized patient guidance and repeated measurements.

In summary, our study demonstrated that intense venous reflux, as detected by presurgical scrotal ultrasound, is associated with impaired semen quality and failure to achieve pregnancy after microsurgical varicocelectomy. This study may provide more prognostic information for patients who undergo surgical repair for varicocele.

AUTHOR CONTRIBUTIONS

KY was in charge of the analysis and interpretation of data and critical revision of the manuscript for important intellectual content. BBC was in charge of the acquisition of data. PW was in charge of technical support and the acquisition of data. RGB was in charge of administrative, technical, or material support and critical revision of the manuscript for important intellectual content. XWX was in charge of study concept and design, drafting of the manuscript, statistical analysis, and supervision. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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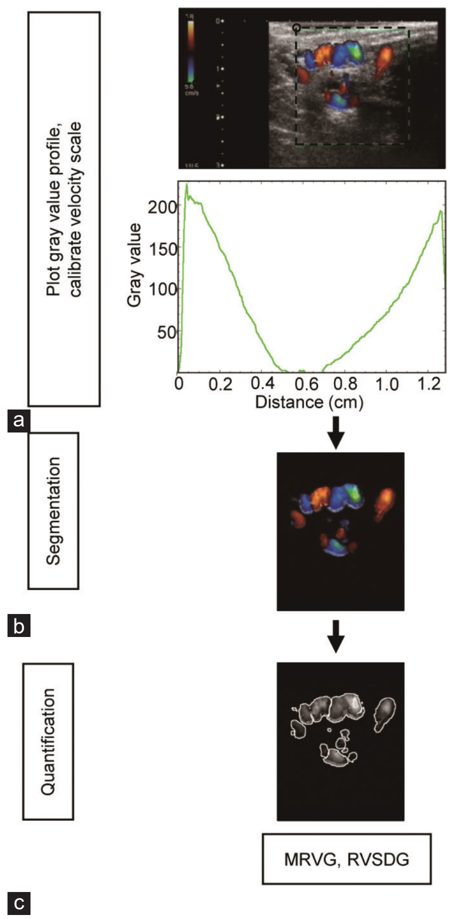
Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

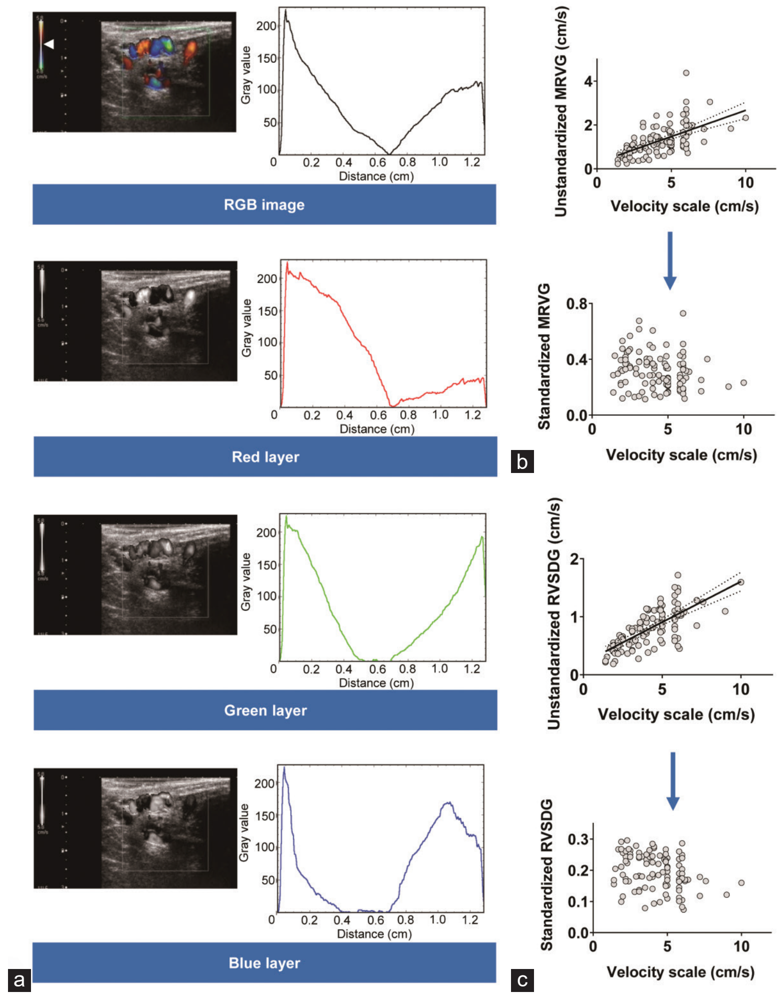
REFERENCES

1 Naughton C, Nangia A, Agarwal A. Pathophysiology of varicoceles in male infertility. *Hum Reprod Update* 2001; 7: 473–81.
2 Bagheri S, Khajehasani F, Iraji H, Fatemi I. A novel method for investigating the role of reflux pattern in color Doppler ultrasound for grading of varicocele. *Sci Rep* 2018; 8: 6517.
3 Jensen C, Ostergren P, Dupree J, Ohl D, Sonksen J, *et al*. Varicocele and male infertility. *Nat Rev Urol* 2017; 14: 523–33.
4 Baazeem A, Belzile E, Ciampi A, Dohle G, Jarvi K, *et al*. Varicocele and male factor infertility treatment: a new meta-analysis and review of the role of varicocele repair. *Eur Urol* 2011; 60: 796–808.
5 Kroese A, De Lange N, Collins J, Evers J. Surgery or embolization for varicoceles in subfertile men. *Cochrane Database Syst Rev* 2012; 10: CD000479.
6 Nieschlag E, Hertle L, Fischedick A, Abshagen K, Behre H. Update on treatment of varicocele: counselling as effective as occlusion of the vena spermatica. *Hum Reprod* 1998; 13: 2147–50.
7 D'Andrea S, Barbonetti A, Castellini C, Nolletti L, Martorella A, *et al*. Left spermatic vein reflux after varicocele repair predicts pregnancies and live births in subfertile couples. *J Endocrinol Invest* 2019; 42: 1215–21.
8 D'Andrea S, Micillo A, Barbonetti A, Giordano A, Carducci S, *et al*. Determination of spermatic vein reflux after varicocele repair helps to define the efficacy of treatment in improving sperm parameters of subfertile men. *J Endocrinol Invest* 2017; 40: 1145–53.
9 Vanlangenhove P, Dhondt E, Everaert K, Defreyne L. Pathophysiology, diagnosis and treatment of varicoceles: a review. *Minerva Urol Nefrol* 2014; 66: 257–82.
10 Vanlangenhove P, Dhondt E, Van Maele G, Van Waesberghe S, Delanghe E, *et al*. Internal spermatic vein insufficiency in varicoceles: a different entity in adults and adolescents? *Am J Roentgenol* 2015; 205: 667–75.
11 Baker S, Burnand K, Sommerville K, Wilson N, Browse N, *et al*. Comparison of venous reflux assessed by duplex scanning and descending phlebography in chronic venous disease. *Lancet* 1993; 341: 400–3.
12 Choi B, Kim T, Han J, Kim A, Seong C, *et al*. Vascularity of hepatocellular carcinoma: assessment with contrast-enhanced second-harmonic versus conventional power Doppler US. *Radiology* 2000; 214: 381–6.
13 Goren M, Erbay G, Ozer C, Kayra M, Hasirci E. Can we predict the outcome of varicocelectomy based on the duration of venous reflux? *Urology* 2016; 88: 81–6.
14 Enatsu N, Yamaguchi K, Chiba K, Miyake H, Fujisawa M. Clinical outcome of microsurgical varicocelectomy in infertile men with severe oligozoospermia. *Urology* 2014; 83: 1071–4.
15 Pham K, Sandlow J. The effect of body mass index on the outcomes of varicocelectomy. *J Urol* 2012; 187: 219–21.
16 Hussein K, Ashok A, Ramadan S, Florence B, Mohamed A, *et al*. Editorial commentary on draft of World Health Organization sixth edition laboratory manual for the examination and processing of human semen. *World J Mens Health* 2021; 39: 577–80.
17 Belay R, Huang G, Shen J, Ko E. Diagnosis of clinical and subclinical varicocele: how has it evolved? *Asian J Androl* 2016; 18: 182–5.
18 Dubin L, Amelar R. Varicocele size and results of varicocelectomy in selected subfertile men with varicocele. *Fertil Steril* 1970; 21: 606–9.
19 Abdel-Meguid T, Al-Sayyad A, Tayib A, Farsi H. Does varicocele repair improve male infertility? An evidence-based perspective from a randomized, controlled trial. *Eur Urol* 2011; 59: 455–61.
20 Mongioì L, Mammino L, Compagnone M, Condorelli R, Basile A, *et al*. Effects of varicocele treatment on sperm conventional parameters: surgical varicocelectomy versus sclerotherapy. *Cardiovasc Intervent Radiol* 2019; 42: 396–404.
21 Pepin F, Julien A, Fugaru I, Lihimdi N, Thariat J, *et al*. Regression rate of choroidal melanoma following iodine-125 brachytherapy is not associated with metastatic spread. *Melanoma Res* 2019; 29: 295–300.
22 Minayoshi K, Okada H, Fujisawa M, Yamasaki K, Kamidono S. Hemodynamic evaluation of left testicular varicocele by scrotal scintigraphy. *Eur Urol* 2001; 39: 30–5.
23 Rehman K, Zaneb H, Qureshi A, Yousaf M, Numan A, *et al*. Correlation between testicular hemodynamic and semen quality indices in clinical varicocele patients in Pakistan. *Biomed Res Int* 2019; 2019: 7934328.

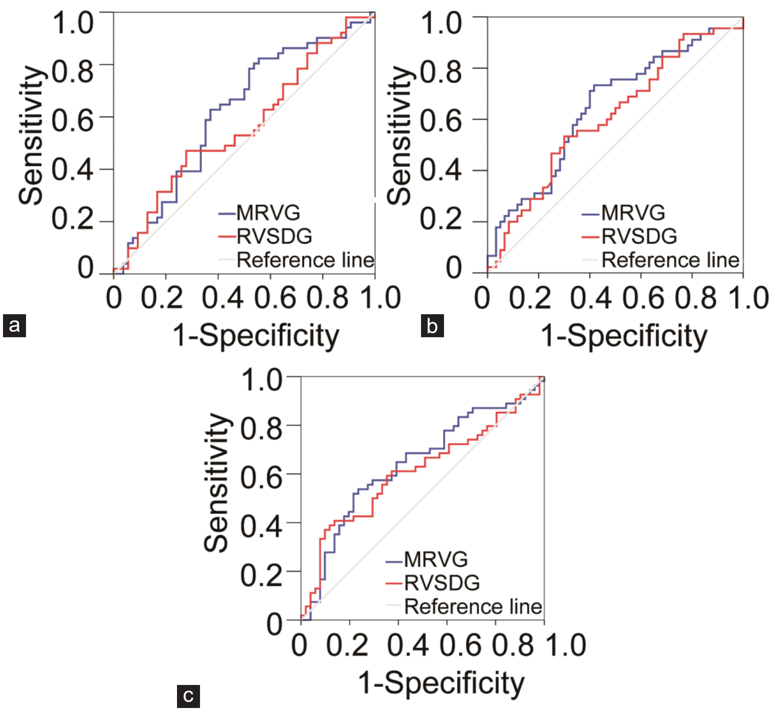
24 Sigmund G, Gall H, Bahren W. Stop-type and shunt-type varicoceles: venographic findings. *Radiology* 1987; 163: 105–10.
25 Ekin E, Kurtul Yildiz H. The importance of great saphenous vein types and segmental aplasia in venous reflux. *J Clin Ultrasound* 2017; 45: 332–6.
26 Lui M, Martino S, Salerno M, Quadrio M. On the turbulence modeling of blood flow in a stenotic vessel. *J Biomech Eng* 2020; 142: 011009.
27 Switzer D, Yoganathan A, Nanda N, Woo Y, Ridgway A. Calibration of color Doppler flow mapping during extreme hemodynamic conditions *in vitro*: a foundation for a reliable quantitative grading system for aortic incompetence. *Circulation* 1987; 75: 837–46.
28 Kocakoc E, Serhatlioglu S, Kiris A, Bozgeyik Z, Ozdemir H, *et al*. Color Doppler sonographic evaluation of inter-relations between diameter, reflux and flow volume of testicular veins in varicocele. *Eur J Radiol* 2003; 47: 251–6.
29 Van Batavia J, Fast A, Nees S, Mercado M, Gaselberti A, *et al*. Incidence, significance and natural history of persistent retrograde venous flow after varicocelectomy in children and adolescents: correlation with catch-up growth. *J Urol* 2013; 190: 689–95.
30 Belloli G, D'Agostino S, Zen F, Ioverno E. Fertility rates after successful correction of varicocele in adolescence and adulthood. *Eur J Pediatr Surg* 1995; 5: 216–8.



Supplementary Figure 1: Image processing and data collection. (**a**) The edge point of the velocity scale was indicated (big blue dots), and the edge point of the region of interest was indicated (big red dots) to plot the gray value profile and calibrate velocity scale. (**b**) Color-only reflux signals were segmented and the black and white background was cleared. (**c**) The reflux signals in the green layer of the RGB stack were selected (white arrow), and the mean reflux velocity of the green layer (MRVG) and the reflux velocity SD of the green layer (RVSDG) were measured. s.d.: standard deviation, MRVG: mean reflux velocity of green layer; RVSDG: reflux velocity standard deviation of green layer.



Supplementary Figure 2: The gray value profile of the velocity scale and standardization of reflux velocity of green layer (MRVG) and reflux velocity SD of green layer (RVSDG). (**a**) Vertical lines (arrow head) overlapping velocity scales in the RGB image as well as the red, green and blue layers of RGB stack, then gray value profiles of velocity scales were plotted. (**b**) Point graphs show the distribution of the unstandardized MRVG, which was standardized by the velocity scale ($n = 106$). Solid lines represent linear regression; dotted lines represent error bars. (**c**) Point graphs show the distribution of the unstandardized RVSDG, which was standardized by the velocity scale ($n = 106$). Solid lines represent linear regression; dotted lines represent error bars. s.d.: standard deviation; MRVG: mean reflux velocity of green layer; RVSDG: reflux velocity standard deviation of green layer.



Supplementary Figure 3: The ROC curves of MRVG and RVSDG were plotted for predicting (**a**) impaired progressive motility, (**b**) impaired sperm concentration, and (**c**) failing to achieve pregnancy. ROC: receiver operating characteristic; MRVG: mean reflux velocity of green layer; RVSDG: reflux velocity standard deviation of green layer.