Am J Transl Res 2021;13(8):9136-9142
www.ajtr.org /ISSN:1943-8141/AJTR0134639

*Original Article*

# Evaluation of testicular spermatogenic function by ultrasound elastography in patients with varicocele-associated infertility

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Received April 25, 2021; Accepted May 31, 2021; Epub August 15, 2021; Published August 30, 2021

**Abstract:** Objective: To investigate the application of ultrasound elastography and color Doppler ultrasound in the evaluation of testicular spermatogenic function in patients with varicocele (VC) associated infertility. Methods: A total of 196 patients with VC-associated infertility treated in our hospital from January 2018 to January 2020 were included as the research subjects (VC group), and were classified into VC I group (66 cases), VC II group (60 cases), and VC III group (70 cases) according to the diameter of spermatic vein and the degree of reflux under ultrasound. 50 age-matched healthy subjects were included as the control group. Color ultrasound, ultrasound elastography and semen examination were performed on all patients. Results: Compared with control group, a lower left testicular volume in VC group and a lower right testicular volume in VC III group were observed, and the left testicular volume was inversely proportional to VC grade (all P<0.05). Statistically significant differences in sperm viability, sperm density, peak systolic velocity (PSV), and MEAN values were determined among the 4 groups by one-way ANOVA, and between two groups by the independent-samples t test (all P<0.05). Pearson correlation analysis revealed that sperm viability and sperm density had no correlation with PSV, resistive index (RI) and pulsatility index (PI) of the left testicular artery in VC group, but were negatively correlated with the MEAN values of the testis. Conclusion: Ultrasound elastography can predict the semen quality in patients with VC-associated infertility. Clinical trial registration: This study was registered with the registration No. ChiCTR2010189 (URL: http://www.chictr.org.cn/showproj.aspx?proj=2018XE057-3).

**Keywords:** Varicocele, infertility, ultrasound elastography, semen quality, correlation analysis

## Introduction

Varicocele (VC), one of the common causes of male infertility, is mainly triggered by abnormal dilatation, elongation and tortuosity of the spermatic vein and the variegated venous plexus, and is characterized by scrotal pain and discomfort, infertility, and testicular atrophy [1-3]. The failure of timely diagnosis and treatment in most cases is in consequence of the rather hidden typical symptoms, resulting in impaired spermatogenesis in some cases. Although semen examination has been long appreciated in the evaluation of sperm quality, its accuracy is critically undermined by individual errors and differences between samples [4]. Color Doppler ultrasound, as the major technique for the diagnosis of VC, can accurately observe the dilatation degree and blood flow state of VC, but cannot give an accurate evaluation of testicular function [5]. Studies have shown that the damage of VC to the testis will give rise to a thickening of the basement membranes of the seminiferous tubules, as well as hypertrophy of collagen fibers and interstitial cells, which leads to an increase in testicular hardness [6]. Ultrasound elastography is an emerging ultrasonic technique that has been widely used in the diagnosis of breast cancer and thyroid cancer to accurately assess changes in tissue stiffness. However, its application in VC has not yet been reported thoroughly [7]. Accordingly, we aimed to explore the role of ultrasound elastography and color ultrasound Doppler in the evaluation of testicular function in patients with VC-associated infertility.

## Materials and methods

### *General data*

196 patients with VC-associated infertility treated in the Andrology Clinic of our hospital from January 2018 to January 2020 were enrolled and divided into VC I (66 cases), VC II (60 cases) and VC III (70 cases) groups according to the diameter of the spermatic vein and the degree of reflux under ultrasound. In addition, 50 age-matched male subjects, who underwent physical examination in our hospital, concurrently, were selected as the control group. The protocol was ethically approved by the Institution Animal Ethics Committee of Affiliated Hospital of Hebei Engineering University (Hebei, China; license no. KY 2018-345C-102).

### *Inclusion and exclusion criteria*

Inclusion criteria: (1) aged from 20 to 45 years old; (2) diagnosed with left VC by ultrasound diagnosis [8]; (3) patients with a history of infertility for 1 to 5 years; (4) patients and their family members signed the informed consent after being fully informed of the purpose and process of the research. Exclusion criteria: Patients with (1) testicular stones, testicular/epididymitis, testicular tumors or other diseases that cause male infertility; (2) history of testicular trauma; (3) congenital testicular malformations or external genital abnormalities, or; (4) diseases such as moderate to large hydrocele and perineal skin lesions that affect the ultrasound examination.

### *Ultrasound method*

*Ultrasound elastography examination:* The HITACHI-HI-VISION Avi-us ultrasound system (Hitachi, Japan) was used with a probe frequency of 7 MHZ and a built-in HITACHI-HI-VISION Avius data processing system.

All subjects underwent color Doppler ultrasound (Hitachi Hivision Preim) to examine the testes. The testis and epididymal spermatic vein were examined by two-dimensional ultrasonography and testicular volume was calculated. Testicular volume = [length (mm) × width (mm) × height (mm)] × 0.71)/1000 (mL). The patient was instructed to lie supine to expose the perineum. Then, the testicular surface was palpated for VC, the inner diameter of the spermatic vein in a calm state was measured, and the reflux was measured by Valsalva maneuver to determine the diagnosis and classification of VC. VC was confirmed if the spermatic vein maximum diameter at rest (DR) ≥1.8 mm and the diameter at Valsalva maneuver (DV) ≥2.0 mm. Among them, VC I is interpreted as DR 0.18-0.20 cm, and a small amount of reflux can be observed by Valsalva; VC II is translated in DR 0.21-0.30 cm, and moderate reflux can be observed by Valsalva; VC III corresponds to DR >0.30 cm, and a large amount of reflux can be observed by Valsalva. Peak systolic velocity (PSV), end-diastolic velocity (EDV), resistance index (RI) and pulsatility index (PI) of the testicular artery were measured by ultrasonic power Doppler. Ultrasound elastography was performed after longitudinal section showing the largest section of the testis. The region of interest covers the testis and surrounding tissues as much as possible, including capsule, skin, and epididymis. The center of the region of interest is defined as the tissue dispersion quantitative analysis region, and the absolute red and absolute blue regions are excluded. The probe was pressed vertically on the surface of the scrotum in a pressurization-depressurization way, with a depth of about 1-2 mm and a frequency of 1-2 times/s; the pressure guide bar was held at 3 or 4, and the image was frozen after the display stabilized, for subsequent measurements. The measurement was repeated 5 times and the average value was obtained, which was calculated as the mean strain of ultrasound elastography (MEAN values). The larger the MEAN value, the softer the tissue. All ultrasound examinations were analyzed by 2 senior sonographers, and relevant image data were retained for further investigation. Typical testicular ultrasound elastography is shown in **Figure 1**.

### *Semen examination*

Standardized semen sampling was performed on all subjects. Each participant was abstinent for 3 to 5 days before sampling, and the semen obtained by masturbation was examined within 30 minutes. Routine sperm inspection includes sperm density and sperm viability.

### *Statistical analysis*

Data analyses were conducted by SPSS 22.0 software. Measured data were expressed as ($\bar{x}$±s), and analyzed by independent sample

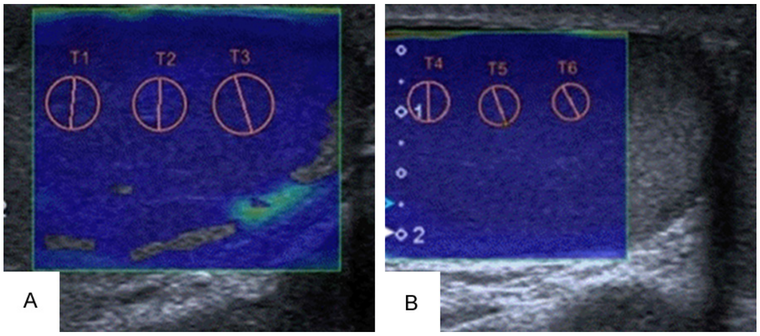


**Figure 1.** Typical figures of testicular ultrasound elastography of left-sided testes. The average stiffness (5.15 kPa) value of the left testis (A) derived from three regions of interest was higher than that of the contralateral (B) testis (4.28 kPa).

**Table 1.** Comparison of general data

| | Age | Bilateral VC | History of infertility |
|---|---|---|---|
| Control group (n=50) | 30.25±8.24 | - | - |
| VC I group (n=66) | 33.17±7.14 | 20 | 5.25±1.54 |
| VC II group (n=60) | 31.21±6.97 | 28 | 5.62±1.37 |
| VC III group (n=70) | 33.25±7.17 | 32 | 5.87±1.75 |
| $F/\chi^2$ | 2.389 | 4.565 | 2.668 |
| P | 0.069 | 0.102 | 0.072 |

t-test or one-way analysis of variance (ANOVA); counted data were expressed as [(n)%], and analyzed by chi-square test. Pearson correlation analysis was used to analyze the correlation. The statistical level was set at α=0.05. P<0.05 was considered significant.

## Results

*Comparison of the general data between VC subgroups and control group*

The age of control group, VC I group, VC II group, and VC III group were (30.25±8.24) years old, (33.17±7.14) years old, (31.21±6.97) years old and (33.25±7.17) years old, respectively (F=2.389, P=0.069). The Bilateral VC cases of VC I group, VC II group, and VC III group were 20/66, 28/60, and 32/70 ($\chi^2$=4.565, P=0.102). The sterile time of VC I group, VC II group, and VC III group were (5.25±1.54) years, (5.62±1.37) years, and (5.87±1.75) years (F=2.668, P=0.072) (**Table 1**).

*Comparison of testicular volume between VC subgroups and control group*

The left-side testicular volume of control group, VC I group, VC II group, and VC III group were (14.15±1.05) mL, (11.35±1.26) mL, (10.42±1.35) mL, and (9.63±1.05) mL; the right-side were (13.98±1.12) mL, (12.03±1.44) mL, (11.74±1.29) mL, and (11.54±1.37) mL, respectively. There was no significant difference between left and right testicular volume in control group. However, notably a lower left testicular volume than that of the right was observed in each VC subgroup. All VC subgroups had lower bilateral testicular volumes, as compared to the control group (P<0.05; **Table 2**).

*Comparison of sperm viability and sperm density between VC subgroups and control group*

The sperm viability of control group, VC I group, VC II group, and VC III group were (54.26±8.25)%, (51.14±9.21)%, (46.23±10.22)% and (34.15±12.74)%, respectively. Sperm densities were (80.58±14.25) $10^6$/mL, (61.22±12.17) $10^6$/mL, (45.62±10.27) $10^6$/mL and (33.17±9.54) $10^6$/mL, respectively. One-way ANOVA showed that there were statistical differences in sperm viability and sperm density among the 4 groups. The sperm viability and sperm density were the highest in the control group, followed in descending order by VC I, VC II and VC III groups (P<0.05; **Table 3**).

*Comparison of left testicular artery PSV, EDV, RI and PI between VC subgroups and control group*

One-way ANOVA revealed a statistical difference in PSV among the 4 groups, but no significant difference in EDV, RI, and PI. The PSV was the lowest in the control group, followed in ascending order by VC I, VC II, and VC III groups (P<0.05; **Table 4**).

*Comparison of mean values of the left testis between VC subgroups and control group*

The mean values of the 4 groups of patients from high to low were VC III group (157.95±14.67), VC II group (148.69±12.97), VC I group (138.26±11.24) and control group

**Table 2.** Comparison of testicular volume in patients with different degrees of varicosity

| | Testicular volume | | t | P |
|---|---|---|---|---|
| | Left | Right | | |
| Control group (n=50) | 14.15±1.05 | 13.98±1.12 | 0.783 | 0.4436 |
| VC I group (n=66) | 11.35±1.26 | 12.03±1.44 | 2.887 | 0.005 |
| VC II group (n=60) | 10.42±1.35 | 11.74±1.29 | 5.476 | <0.001 |
| VC III group (n=70) | 9.63±1.05 | 11.54±1.37 | 9.258 | <0.001 |
| F | 152.8 | 38.71 | | |
| P | <0.001 | <0.001 | | |

**Table 3.** Comparison of sperm viability and sperm density between VC subgroups and control group

| | Sperm viability (%) | Sperm density ($\times 10^6$/mL) |
|---|---|---|
| Control group (n=50) | 54.26±8.25 | 80.58±14.25 |
| VC I group (n=66) | 51.14±9.21 | 61.22±12.17 |
| VC II group (n=60) | 46.23±10.22 | 45.62±10.27 |
| VC III group (n=70) | 34.15±12.74 | 33.17±9.54 |
| F | 46.08 | 184.20 |
| P | <0.001 | <0.001 |

**Table 4.** Comparison of left testicular artery PSV, EDV, RI and PI between VC subgroups and control group

| | PSV (cm/s) | EDV (cm/s) | RI | PI |
|---|---|---|---|---|
| Control group (n=50) | 8.11±1.24 | 3.46±0.87 | 0.55±0.12 | 0.93±0.24 |
| VC I group (n=66) | 8.65±1.45 | 3.28±0.71 | 0.57±0.07 | 0.96±0.20 |
| VC II group (n=60) | 9.16±1.35 | 3.44±0.79 | 0.61±0.18 | 1.01±0.22 |
| VC III group (n=70) | 10.24±2.07 | 3.18±0.76 | 0.59±0.12 | 1.05±0.34 |
| F | 20.13 | 1.822 | 2.302 | 2.411 |
| P | <0.001 | 0.144 | 0.078 | 0.068 |

PSV = Peak systolic velocity, EDV = end-diastolic velocity, RI = resistance index and PI = pulsatility index

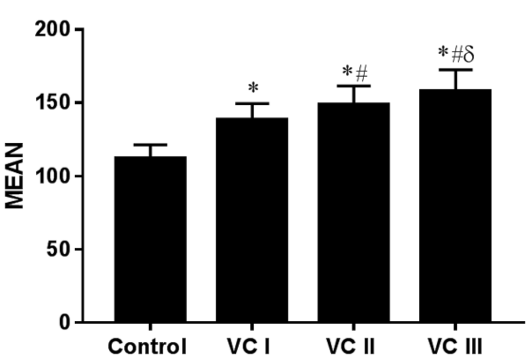


**Figure 2.** Comparison of mean values of the left testis between VC subgroups and control group. * means that the difference is statistically significant compared with the control group; # means that the difference is statistically significant compared with the VC I group; δ represents that the difference is statistically significant compared with the VC II group.

(112.13±9.25). One-way ANOVA detected statistical differences among the 4 groups as well as between groups in terms of mean values (P<0.05; **Figure 2**).

*Correlation analysis of left testicular artery PSV, EDV, PI, RI and mean with sperm viability and sperm density in VC group*

Pearson correlation analysis showed that sperm viability and sperm density had no correlation with PSV (r=0.271, -0.077; P=0.078, 0.563; **Table 5**), EDV (r=0.292, -0.074; P=0.081, 0.612; **Table 6**), PI (r=-0.055, -0.068; P=0.641, 0.571; **Table 7**), or RI (r=-0.314, 0.226; P=0.057, 0.419; **Table 8**). However, results showed that the mean values were significantly correlated with sperm viability and sperm density (r=-0.678, -0.612; P=0.014, 0.032; **Table 9**).

## Discussion

Varicocele (VC) is a common male reproductive problem, that can lead to decreased sperm motility and sperm density and is one of the leading causes of male infertility. Accurate assessment of testicular spermatogenesis plays a pivotal role in accurately reflecting testicular function. Nevertheless, apart from its time-consuming nature, the results of semen examination are easily affected by the environmental factors, emotions, transportation, and preservation conditions during sperm retrieval [9-11]. Additionally, CT and nuclide are excluded for VC as a consequence of the sensitivity of the reproductive system [12]. Color ultrasound can accurately measure morphologic and hemodynamic changes such as testicular volume and microcirculation; however, the relationship between ultrasound-related indicators and sperm quality is elusive [13]. With the advancement of ultrasound technology, ultrasound elastography can non-invasively measure tis-

**Table 5.** Correlation analysis of PSV with sperm viability and sperm density

| | Sperm viability | | Sperm density | |
|---|---|---|---|---|
| | r | P | r | P |
| PSV | 0.271 | 0.078 | -0.077 | 0.563 |

**Table 6.** Correlation analysis of EDV with sperm viability and sperm density

| | Sperm viability | | Sperm density | |
|---|---|---|---|---|
| | r | P | r | P |
| EDV | 0.292 | 0.081 | -0.074 | 0.612 |

**Table 7.** Correlation analysis of PI with sperm viability and sperm density

| | Sperm viability | | Sperm density | |
|---|---|---|---|---|
| | r | P | r | P |
| PI | -0.055 | 0.641 | 0.068 | 0.571 |

**Table 8.** Correlation analysis of RI with sperm viability and sperm density

| | Sperm viability | | Sperm density | |
|---|---|---|---|---|
| | r | P | r | P |
| RI | -0.314 | 0.057 | 0.226 | 0.419 |

**Table 9.** Correlation analysis of mean values with sperm viability and sperm density

| | Sperm viability | | Sperm density | |
|---|---|---|---|---|
| | r | P | r | P |
| MEAN | -0.678 | 0.014 | -0.612 | 0.032 |

sue hardness, which has been considered as a new ultrasound technique following gray-scale ultrasound, two-dimensional ultrasound, and three-dimensional ultrasound, greatly expanding the application range of ultrasound [14]. Due to its non-invasive, simple, reproducible, and cost-effective characteristics, ultrasound elastography has been widely used in the identification of benign and malignant tumors of the thyroid, prostate, liver and other tissues with higher sensitivity and specificity [15].

It has been confirmed that VC can cause male infertility. At present, there are many explanations about its mechanism of triggering male infertility and testicular pathologic changes, including gonadal hormone suppression, toxin reflux, and local temperature increase. Consequently, the spermatogenic function of the testis is reduced by a cascade of possible mechanisms, which eventually leads to poor semen quality and infertility. Studies have pointed out that the more severe the VC, the more affected the semen quality of the patient, which is manifested by changes in sperm morphology, reduced number, and decreased vitality. The results of this study showed that the volume of the left testis in VC group was lower than that of the control group and the right testis. The right testicular volume in V III group was drastically lower than that in control group. In addition, with the increase of VC grade, the left testicular volume witnessed a decline. Also, there were significant differences in sperm viability and sperm density among the 4 groups. The sperm viability and sperm density from low to high were VC III group, VC II group, VC I group, and control group, which was consistent with prior studies. Testicular volume and sperm quality are closely related, and about 80% of the testicular tissue is composed of seminiferous tubules [16]. The decrease in testicular volume and sperm quality caused by VC is well-known.

To the best of our knowledge, VC can induce microcirculation disorders, which will affect the blood supply of the testis, leading to sperm motility decline and maturation disorders. Left VC can cause left renal vein reflux and result in related metabolic products refluxing into the testis, causing testicular damage [17, 18]. In this study, there were significant differences in PSV between the VC group and control group as well as among VC subgroups. According to Pearson correlation analysis, PSV had no correlation with sperm viability and sperm density. It has been proven that an increase in PSV indicates an increase in blood flow per unit time, which can cause an elevation in testicular temperature. The spermatogenesis of the testis requires a relatively constant temperature of 1°C lower than the body temperature, while PSV increases significantly during VC, which may be one of the causes of infertility [19, 20]. To date, however, the mechanism of the increase in PSV is poorly understood, and the relationship between VC, PSV, and sperm quality needs further research. Relevant studies have shown that VC can cause progressive damage to the structure and function of the testis over time, and the quality of semen is also affected by the course of the disease.

Consequently, a full assessment of sperm quality requires more evidence than sperm motility and sperm density.

In this study, the mean values of each group were statistically different, and were negatively correlated with sperm viability and sperm density. The more severe the VC, the smaller the size of the testicles and the lower quality of sperm. VC is known to cause pathologic changes in the testis, including thickening and degeneration of the basement membrane of seminiferous tubules. Testicular stromal cell deformation and karyopyknosis will seriously affect sperm quality and testicular texture. Prior studies have shown that as the VC level increases, the mean strain of the testis elevates correspondingly, and the sperm density observes a downturn. The mean strain of the testis is negatively correlated with sperm density, which is consistent with the results of this study.

This study employed elastic ultrasound imaging technology to non-invasively and in real-time, assess the quality of the patient's testicles, and evaluate the quality of semen, which has high application value for the treatment of patients with VC and infertility.

Nonetheless, this study is limited by the following shortcomings: Firstly, in this study, only one semen collection of each patient was carried out, which resulted in a deficiency of some indicators such as sperm survival rate and sperm deformity. Therefore, it was virtually impossible to get complete assessment of semen quality. The application and research of ultrasound elastography to evaluate the texture of the testis are minimal, and there is no uniform standard for the operation method.

In summary, the mean ultrasound elastography result using a new generation of ultrasound technology is significantly correlated with sperm viability and sperm density, which can serve as a predictor of semen quality in patients with VC-associated infertility.

## Disclosure of conflict of interest

None.

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