

Original Article

Blood perfusion of the contralateral testis evaluated with contrast-enhanced ultrasound in rabbits with unilateral testicular torsion

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Abstract

The changes of blood perfusion of contralateral testis after unilateral testicular torsion remain controversial. In this study, 28 New Zealand white male rabbits were randomly divided into five groups. Group A ($n = 8$), the control group, underwent a sham operation on the unilateral testis without inducing testicular torsion. In groups B, C, and D ($n = 5$ each), unilateral testicular torsion was induced, and, after 3, 6 or 24 h, respectively, detorsion was performed. In group E ($n = 5$), permanent unilateral testicular torsion was applied. Contrast-enhanced ultrasound was used to observe the blood perfusion of the contralateral testis at the following stages: pre-torsion (preoperation), immediately post-torsion (postoperation), pre-detorsion, immediately post-detorsion, and late-stage post-detorsion (6–12 h post-detorsion in groups B–D) or at a similar time point (15–21 h post-torsion in group E). Time-intensity curves were generated, and the following parameters were derived and analyzed: arrival time, time to peak intensity, peak intensity, and half-time of the descending peak intensity. The analysis revealed that blood perfusion of the contralateral testis increased immediately after testicular torsion on the opposite side ($P < 0.05$), which increased with prolonged testicular torsion of the other testis. This research demonstrated that contrast-enhanced ultrasound was valuable in evaluating blood perfusion of the contralateral testis after unilateral testicular torsion.

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1 Introduction

Testicular torsion, also called spermatic cord torsion, is a medical emergency that mainly affects

young men, especially during puberty [1]. Since the very first report of testicular torsion by Delasiave in 1810, numerous studies have been performed on this pathological condition. However, most earlier studies have focused only on the ipsilateral testis. In recent years, clinical and experimental studies have suggested that the injured testis may result in damage to the contralateral testis after unilateral testicular torsion [2, 3]. Hormonal, biochemical, immunological, histopathological, and vasculogenic studies have been performed, but the results are still

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controversial. Different mechanisms have been proposed for this phenomenon, including ischemia, hypoxia [4], and activation of oxygen-free radicals caused by reperfusion [5].

However, the specific mechanisms still need to be clarified. Although unilateral testicular torsion generally requires emergent intervention, no matter what the influence on the contralateral testis, it is important to evaluate the blood perfusion of the contralateral testis for potential damage. This issue can be examined using noninvasive imaging technology such as ultrasound. In an earlier study on dogs with unilateral testicular torsion, Tarhan *et al.* [6] used color Doppler ultrasound and found no significant changes in the blood supply of the contralateral testis. However, recent studies have shown that contrast-enhanced ultrasound is highly sensitive for detecting slowed blood flow in parenchymal organs [7]. Therefore, in this study, we used contrast-enhanced ultrasound to evaluate the changes in blood perfusion of the contralateral testis following unilateral testicular torsion of rabbits. We also used this study to explore the potential usefulness of this ultrasound for clinical practice.

2 Materials and methods

2.1 Animals

A total of 28 New Zealand white male rabbits, weighing 2.6–3.2 kg, were obtained from the Field of Laboratory Animals (Songjiang District, Shanghai, China). They were maintained on a standard pellet diet with tap water *ad libitum* and kept in stainless steel cages with woodchip bedding under a 12 h:12 h light:dark cycle and a room temperature of 22–24°C. All the animals were allowed to acclimate to the laboratory environment for 2 weeks before the experiment. The experimental protocol was approved by the Animal Ethics Committee in accordance with the guide for the care and use of laboratory animals established by the Medical School of Shanghai Jiao Tong University (Shanghai, China).

2.2 Ultrasound imaging system and settings

An ultrasound system, Mylab 30 (Esaote, Italy), with a linear probe that transmits and receives center frequencies of 7.5 MHz, was used for contrast-enhanced ultrasound image acquisition. The mechanical index was set at 0.09. Gain and sensitivity time control were set at optimal levels, and the focal point was adjusted to the middle level of the image. The instrumental

settings were kept identical for all experiments.

2.3 Contrast agent

SonoVue (Bracco S.p.A., Milan, Italy) contrast agent was used in this study. The active component of SonoVue is sulfur hexafluoride, in the form of a white powder. After the powder was shaken with 5.0 mL of a 0.9% saline solution, the microbubble suspension was injected as a bolus into the rabbits through an ear vein at a dose of 0.1 mL kg⁻¹ and immediately flushed with 1.0 mL of saline solution.

2.4 Anesthesia

All the rabbits were anesthetized through an intra-muscular injection using haloperidol at a dose of 0.2 mL kg⁻¹; the total dose for any rabbit did not exceed 1.0 mL.

2.5 Experimental protocol

The rabbits were randomly divided into five groups. Group A contained eight animals, which underwent a sham operation on the unilateral testis without inducing testicular torsion. Group B had five animals in which unilateral testicular torsion was induced and detorsion performed after 3 h. Group C comprised five animals in which detorsion was conducted after 6 h of testicular torsion. Group D contained five animals in which detorsion was conducted after 24 h of testicular torsion, and five animals in group E had permanent unilateral testicular torsion.

All surgeries were performed by one urologist (WBR) while the animals were completely anesthetized. The region of operation was shaved and sterilized using povidone iodine. In group A, one side of the scrotum was incised from the dorsal side down to the tunica vaginalis. The testis was released and then immediately returned, and the incision was sutured with 3/0 silk. In groups B–E, the testis was released in the same way, but it underwent torsion for different periods of time (or permanently), as stated above. The testis was twisted around the spermatic cord to 720–2 520° (depending on the dramatic diversity in the length of spermatic cords) and fixed to the scrotum with 3/0 silk. The incisions were sutured. Generally, the testis became purple post-torsion, and no blood flow signals could be detected using color Doppler flow imaging. After the designed time of torsion in groups B–D, detorsion was conducted. However, in group E, the twisted testis was not restored, and the torsion was made permanent.

2.6 Contrast-enhanced ultrasound imaging

When the unilateral testis was sham-operated or underwent torsion, the first author (LC) used real-time contrast-enhanced ultrasound imaging to observe the blood perfusion of the contralateral testis. To ensure consistency, the maximum transverse sections of all the contralateral testes in the study were obtained. In group A, ultrasound imaging was conducted just before and immediately after the sham operation. In groups B–D, ultrasound imaging was conducted at the following time points: (1) just before unilateral testicular torsion; (2) immediately after torsion; (3) just before detorsion; (4) immediately after detorsion; and (5) at a late stage (6–12 h) after detorsion. In group E, ultrasound imaging was conducted just before the unilateral testicular torsion, immediately after torsion, and 15–21 h after torsion. At each time point, the imaging data acquisition lasted for at least 3 min following injection of the contrast agent, and the obtained grayscale imaging sequences were stored in the database.

2.7 Image analysis

All the imaging sequences were analyzed independently by two double-blinded readers (CL and PZ) using DFY software, version 2.0 (Institute of Ultrasound Image, Chongqing Medical College, Chongqing, China). The time-intensity curves (TICs) were derived from the imaging sequences, and the regions of interests (ROIs) were manually placed according to the size and shape of the contralateral testes. The TICs allow for the measurement of many parameters. First, the arriving time (AT) of the contrast agent is the time from the injection of the agent to the point where the image intensity increases to 120% of the basal level. Second, the time to peak intensity (TTP) is the time it takes for the image intensity to reach the maximum from the injection of the agent. Third, the peak intensity (PI) is the maximal intensity on the TIC. Lastly, the half-time of descending (HT) is the time from the injection of the agent to the point at which the intensity decreases to half of the maximal intensity. The average values of the two groups from the two radiologists were used for statistical analyses, which were performed by the first author.

2.8 Statistical analysis

The data were analyzed using SPSS 13.0 (SPSS, Chicago, IL, USA). All presented data are expressed as mean \pm SD. Dunnett's *t*-test was used to detect differences between just before and immediately after

the sham operation in group A and between just before and immediately after torsion in groups B–E. Analysis of variance was used to detect the differences among the torsion groups. $P < 0.05$ was considered significant.

3 Results

The perfusion parameters, AT, TTP, HT, and PI, are shown in Table 1 for group A, before and after the sham operation. There were no significant differences ($P > 0.05$) in those measurements between pre- and post-operation stages (Figure 1). With all animals from groups B–E being analyzed together (Table 2, Figure 2), the blood perfusion of the contralateral testis increased immediately after unilateral testicular torsion. This change in perfusion can be shown by a significant rise of PI ($P < 0.05$). The AT, TTP, and HT also changed slightly but not significantly ($P > 0.05$).

In groups B–E, the dynamic changes of the perfusion parameters, AT, TTP, HT and PI, were analyzed (Tables 3–6, Figures 1–4). The results show that longer times of unilateral testicular torsion led to more obvious increases in blood perfusion of the contralateral testis (Figure 4). The PIs in groups D and E were significantly higher than those in group A ($P < 0.05$), and the PIs in group E at the late-stage post-torsion were significantly higher than the corresponding values in group B ($P < 0.05$). It can be suggested that blood perfusion of the contralateral testis increases with the duration of unilateral testicular torsion.

4 Discussion

This study confirms that unilateral scrotum surgery without inducing testicular torsion has no effect on the blood perfusion of the contralateral testis. In groups with unilateral testicular torsion, the blood perfusion of the contralateral testis was augmented immediately after torsion and continued to increase when the testicular torsion on the opposite side was prolonged.

In an earlier pathological study on the contralateral testis following unilateral testicular torsion [8–10], Hadziselimovic *et al.* [8] found apoptosis of germ cells, uneven distribution of the seminiferous tubules, and extensively reduced numbers of spermatogenic cells. Rodriguez *et al.* [11] studied the immunohistopathological changes in the contralateral testis of rats after spermatic cord torsion, and the results suggested humoral and cellular immune-mediated testicular cell damage of the contralateral testis. Salman

Table 1. Blood perfusion measurements from the contralateral testis of group A, which comprised eight rabbits that underwent sham surgery on one testis (mean \pm SD).

Stage	Cases	AT (s)	TTP (s)	PI (dB)	HT (s)
Pre-operation	8	18.84 \pm 3.70	30.54 \pm 4.98	66.80 \pm 12.64	58.02 \pm 3.76
Immediately post-operation	8	20.86 \pm 3.82	33.30 \pm 3.81	70.80 \pm 6.22	60.40 \pm 5.43

Abbreviations: AT, arriving time; HT, half-time of descending; PI, peak intensity; TTP, time to peak intensity.

Table 2. Blood perfusion measurements from the contralateral testis before and immediately after unilateral testis torsion in all rabbits from groups B, C, D, and E (mean \pm SD).

Stage	Cases	AT (s)	TTP (s)	PI (dB)	HT (s)
Pre-torsion	20	19.06 \pm 3.28	32.25 \pm 6.25	53.85 \pm 11.80	53.76 \pm 11.91
Immediately post-torsion	20	20.35 \pm 3.25	34.85 \pm 6.90	65.25 \pm 10.29*	62.40 \pm 17.71

Abbreviations: AT, arriving time; HT, half-time of descending; PI, peak intensity; TTP, time to peak intensity.

*Significantly different from the corresponding value before testis torsion ($P < 0.05$).

Table 3. The perfusion parameter, AT(s), measured from the contralateral testis when another testis is at one of the following stages: pre-torsion, immediately post-torsion, pre-detorsion, immediately post-detorsion, and late stage (6–12 h post-detorsion in groups B–D and a similar time point in group E) (mean \pm SD).

Group	Cases	Pre-torsion	Immediately post-torsion	Pre-detorsion	Immediately post-detorsion	Late stage
B	5	19.32 \pm 2.25	21.32 \pm 2.23	23.10 \pm 4.74	23.02 \pm 2.95	18.56 \pm 2.34
C	5	18.98 \pm 5.63	20.20 \pm 5.40	22.90 \pm 5.48	24.66 \pm 4.59	18.40 \pm 5.06
D	5	19.54 \pm 1.39	18.92 \pm 1.62	21.96 \pm 3.26	21.70 \pm 3.58	18.28 \pm 3.66
E	5	18.60 \pm 3.47	20.96 \pm 3.05			17.18 \pm 3.54

Abbreviation: AT, arriving time.

Table 4. The perfusion parameter, TTP(s), measured from the contralateral testis when another testis is at one of the following stages: pre-torsion, immediately post-torsion, pre-detorsion, immediately post-detorsion, and delay stage (6–12 h post-detorsion in groups B–D and a similar time point in group E) (mean \pm SD).

Group	Cases	Pre-torsion	Immediately post-torsion	Pre-detorsion	Immediately post-detorsion	Late stage
B	5	33.92 \pm 7.83	34.94 \pm 8.51	40.16 \pm 8.29	42.42 \pm 8.46	41.60 \pm 11.85
C	5	32.70 \pm 5.60	36.54 \pm 6.19	39.68 \pm 7.23	45.46 \pm 4.22	42.22 \pm 10.61
D	5	28.92 \pm 1.63	31.84 \pm 2.86	34.32 \pm 8.34	37.92 \pm 5.84	36.62 \pm 3.99
E	5	30.92 \pm 4.13	32.38 \pm 3.55			31.62 \pm 4.35

Abbreviation: TTP, time to peak intensity.

Table 5. The perfusion parameter, HT(s), measured from the contralateral testis when another testis is at one of the following stages: pre-torsion, immediately post-torsion, pre-detorsion, immediately post-detorsion, and delay stage (6–12 h post-detorsion in groups B–D and a similar time point in group E) (mean \pm SD).

Group	Cases	Pre-torsion	Immediately post-torsion	Pre-detorsion	Immediately post-detorsion	Late stage
B	5	54.42 \pm 12.67	53.30 \pm 20.51	68.96 \pm 17.39	72.26 \pm 22.50	66.94 \pm 18.18
C	5	55.68 \pm 8.22	61.12 \pm 18.28	71.40 \pm 10.88	88.28 \pm 20.57	73.02 \pm 27.46
D	5	51.12 \pm 15.45	61.06 \pm 28.84	63.54 \pm 21.42	57.76 \pm 23.72	75.60 \pm 31.16
E	5	51.48 \pm 11.04	61.68 \pm 10.77			68.52 \pm 8.69

Abbreviation: HT, half-time of descending.

Table 6. The perfusion parameter, PI (dB), measured from the contralateral testis when another testis is at one of the following stages: pre-torsion, immediately post-torsion, pre-detorsion, immediately post-detorsion, and late stage (6–12 h post-detorsion in groups B–D and a similar time point in group E) (mean ± SD).

Group	Cases	Pre-torsion	Immediately post-torsion	Pre-detorsion	Immediately post-detorsion	Late stage
B	5	52.40 ± 12.07	65.40 ± 9.24	72.60 ± 13.28	61.00 ± 11.20	58.60 ± 6.66
C	5	57.60 ± 12.99	64.00 ± 16.84	76.20 ± 12.58	63.20 ± 8.59	59.80 ± 11.71
D	5	56.20 ± 13.44	67.20 ± 6.06	79.60 ± 7.09	70.00 ± 5.79	71.20 ± 5.40 ^a
E	5	49.20 ± 10.57	64.40 ± 9.45			73.80 ± 9.42 ^b

Abbreviation: PI, peak intensity.

^aAt the late stage, the PIs of group D are significantly higher than the corresponding values in group B ($P < 0.05$).

^bAdditionally, the PI of group E is significantly higher than the corresponding values in groups B and C ($P < 0.05$).

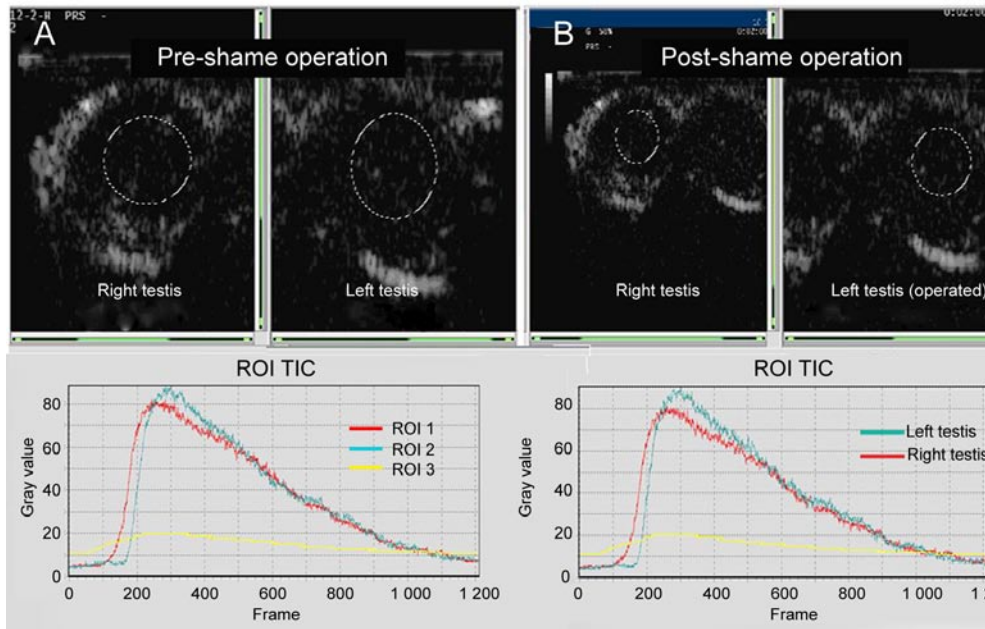


Figure 1. Typical contrast-enhanced ultrasound images and the derived TICs in group A before (A) and immediately after (B) the sham operation. The blue curve represents the perfusion of the operated testis (the left one), and the red curve represents the perfusion of the contralateral testis (the right one). No difference was observed in the peak intensity of the contralateral testis before and immediately after the sham operation (80 dB vs. 82 dB). ROI, regions of interest; TIC, time-intensity curve.

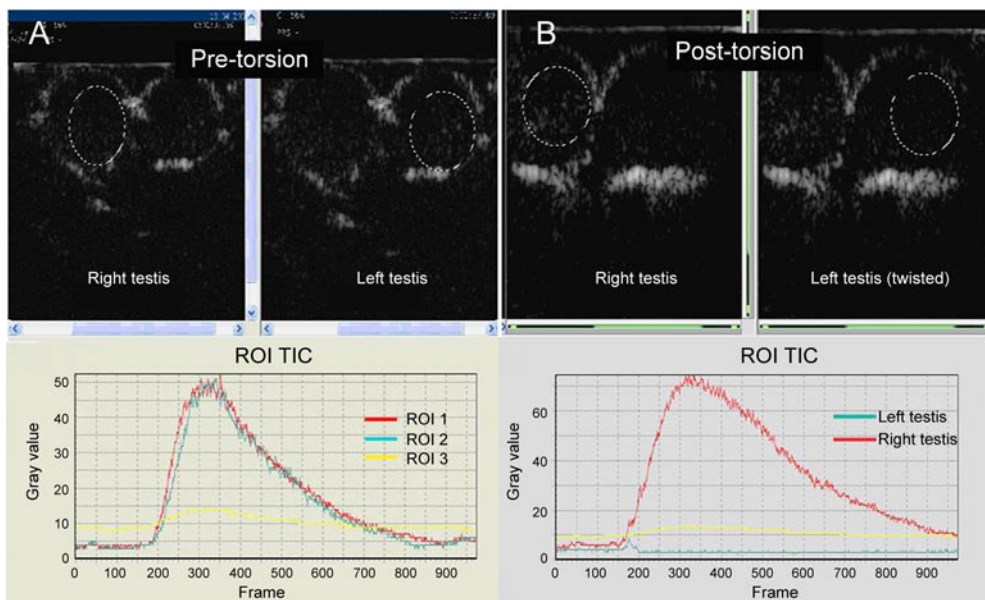


Figure 2. Typical contrast-enhanced ultrasound images and the derived TICs before (A) and immediately after (B) testicular torsion. The blue curves represent the perfusion of the testis with torsion (the left one), and the red curves represent the perfusion of the contralateral testis (the right one). Significant changes were detected in the peak intensity of the contralateral testis, which was 52 dB before and 74 dB after testicular torsion on the opposite side. ROI, regions of interest; TIC, time-intensity curve.

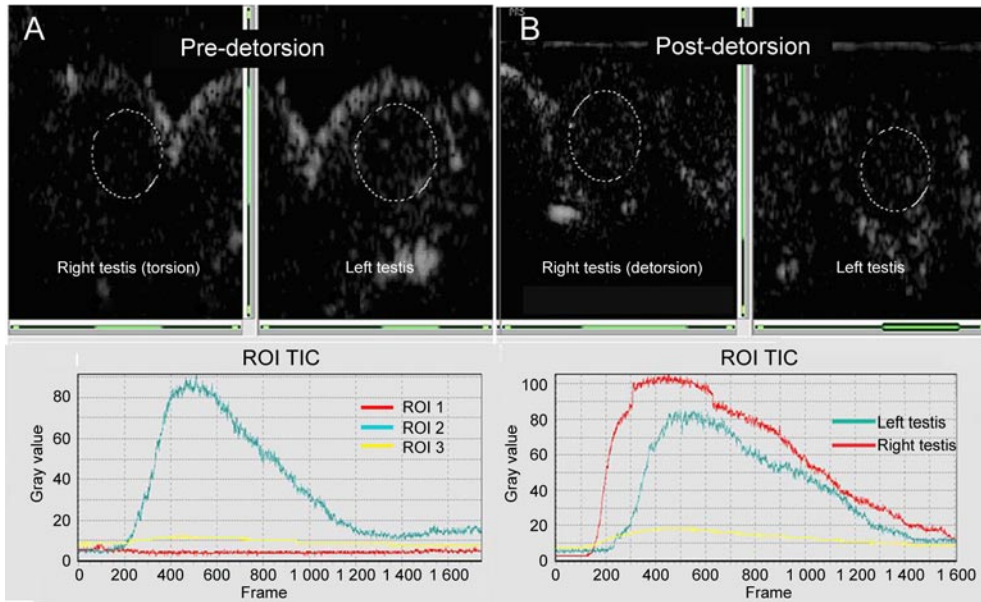


Figure 3. Typical contrast-enhanced ultrasound images and the derived TICs before testicular torsion (A) and immediately after testicular torsion (B). The red curves represent the perfusion of the testis with torsion (right one), and the blue curves represent the perfusion of the contralateral testis (left one). Slight, but not significant, change was observed in the peak intensity of the contralateral testis, which was 91 dB before and 83 dB after testicular torsion on the opposite side. ROI, regions of interest; TIC, time-intensity curve.

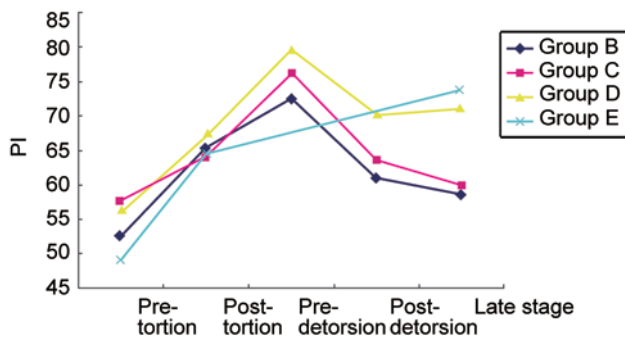


Figure 4. Dynamic changes in the peak intensity (PI) of the contralateral testis in groups (B, C, D, and E) with unilateral testicular torsion and/or detorsion throughout the observed stages. The PI in groups B, C, and D continued to increase from pre-torsion, to immediately post-torsion, and further to pre-detorsion. However, the PI started to decrease immediately after detorsion. The PI continued to decrease into the late stage of groups B and C, but it stayed high in group D. The PI of the contralateral testis in group E increased from the time of pre-torsion to the late stage of the permanent testicular torsion on the opposite side.

et al. [4] suggested that, following unilateral testicular torsion, the blood flow in the contralateral testis slowed through the mediation of body fluids, causing ischemia, hypoxia, and the subsequent pathological changes stated above. However, differing from earlier reports,

we found that after unilateral testicular torsion blood perfusion in the contralateral testis was increased, rather than reduced. This finding was confirmed by the reports from Melikoglu *et al.* [12] and Tanyel *et al.* [13]. Paltiel *et al.* [14] studied perfusion of the contralateral testis in a rabbit model of acute testicular ischemia, which also indicated that radiolabeled microsphere-based perfusion of the contralateral testis increased after unilateral spermatic cord occlusion. We assume that nerve reflex might be involved in this pathophysiological change, as the testis is controlled by the visceral sympathetic nerves [9], and testicular parenchyma exhibit very rich innervation, distributed mainly to blood vessels [15]. The visceral sympathetic nerves can be activated after unilateral testicular torsion and may initiate a series of unknown processes that cause an increase of blood perfusion in the contralateral testis.

Tarhan *et al.* [6] used color Doppler ultrasound to observe blood flow in the contralateral testis after 4 h of unilateral testicular torsion in mature dogs, but they found no significant changes in the blood supply. This result may be because of the limited sensitivity of the color Doppler ultrasound in detecting blood flow, especially in small blood vessels with low velocity [16]. Compared with conventional Doppler ultrasound methods, the contrast-enhanced ultrasound used in this study is more sensitive in detecting blood flow in the testis and in quantifying mild changes of the blood perfusion in microcirculation. The ultrasound contrast

agent consists of microbubbles and a stable shell, which work like scattering bodies in a blood pool [17]. The diameter of the microbubbles is about 2–10 μm (average 2.5 μm). SonoVue does not diffuse into the extravessel compartment. It remains within the blood vessels until the gas dissolves and is then eliminated through the expired air [7]. Under a low mechanical index, the microbubbles are stable and generate strong second harmonic signals. By selectively picking up these harmonic signals, low mechanical index techniques provide real-time images almost exclusively by the microbubble signal (the tissue signal is virtually cancelled) [18]. The signals from the microbubbles in the blood flow are therefore enhanced dramatically [19]. Thus, blood flowing with a very low velocity, such as in the microcirculation of the testis, can be detected, and the slightest change in blood perfusion in the contralateral testis can be quantitatively measured with the TICs [20].

As we used a new method to study perfusion of the contralateral testis after unilateral testicular torsion, there is still a noticeable limitation in our study; namely, we were restricted by imaging in a single testicular tissue plane [13]. The TIC and the parameters, including AT, TTP, HT and PI, were generated from the image of a single tissue plane, which was supposed to be representative of the entire organ. In fact, the values from a single tissue plane were variable, and there was a need for volumetric flow information to more accurately determine perfusion in the testis as a whole [14]. However, current scrotal imaging devices do not permit simultaneous acquisition of perfusion data from multiple tissue planes [14]. Averaging the data obtained from the same tissue plane to each testis would result in an improved estimate of total organ perfusion. Thus, data for the maximum transverse section were obtained for all the testes in the study.

Although an increase in blood perfusion in the contralateral testis was observed in this study after unilateral testicular torsion, the mechanism related to the long-term injury of the contralateral testis is still unclear. Lysiak *et al.* [5] studied ischemia/reperfusion injury to the testis in a mouse model and found that reperfusion resulted in a significant number of active oxygen-free radicals. Normal testicular tissue could be defended from injury by active oxygen-free radicals, but in the condition of unilateral testicular torsion, the defense capabilities of the testis declined, and damage in the contralateral testis inevitably occurred. Thus,

we hypothesize that multiple factors contribute to the injury of the contralateral testis after unilateral testicular torsion. In our study, we specifically confirmed injury to the contralateral testis associated with an increase in blood perfusion.

On the basis of the current results, we can safely draw the conclusion that contrast-enhanced ultrasound is valuable in evaluating blood perfusion in the contralateral testis after unilateral testicular torsion. This study also suggests a potential application for the contrast-enhanced ultrasound in clinical practice. However, further studies are needed to elucidate the mechanism of the damage to the contralateral testis following unilateral testicular torsion.

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