

The effectiveness of extra-scrotal fixation following manual detorsion for testicular torsion: a pilot study in a rabbit model

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Introduction To investigate the effectiveness of manual detorsion (MD) and applicability of extra-scrotal fixation for testicular torsion in a rabbit model.

Material and methods Twelve New Zealand male rabbits were randomized into six groups of two rabbits each. A single-side testicular torsion (TT) model (different degrees, time and sides) was performed in all groups except the Sham group. The groups included: Group 1 (180°; 4 h), Group 2 (720°; 6 h), Group 3 (1080°; 9 h), Group 4 (540°; 1 h), Group 5 (900°; 2 h), and Group 6 (sham-only). Testes were examined by another urologist and radiologist with Color Doppler Ultrasonography (CDU). MD was performed with CDU until blood flow was observed in the affected testis.

Extra-scrotal fixation was then conducted in these animals. The testes were then harvested for blinded histopathological examinations.

Results TT was detected in all animals except the control group. The CDU examination detected decreased blood flow only in Group 1. An opposite rate was observed between the spermatic cord diameter and torsion degree. A wrong direction of MD in the first step was observed in two rabbits in Groups 4 and 5. Torsion signs were observed only in Group 3. Rest torsion was observed in Groups 3 and 5 after extra-scrotal fixation. Histopathological examinations showed that testicular damage increased in parallel to torsion duration.

Conclusions Extra-scrotal fixation after MD along with CDU may be a simple and minimally invasive treatment option in TT therapy. However, this must be verified with further studies.

Key Words: extra-scrotal fixation ◊ Doppler ultrasonography ◊ manual detorsion
◊ scrotal exploration ◊ testicular torsion

INTRODUCTION

It is currently possible to make a diagnosis with almost 100% accuracy for testicular torsion (TT) on physical examination with the help of using colour Doppler ultrasonography (CDU) [1–5]. Manual detorsion (MD) in a TT case is a way of simple and non-invasive management to save the testicles. Testicular fixation is recommended to prevent intermittent torsion, since MD has not been a enough therapy, even in the cases of successful MD [6]. Although many successful results have been reported [7–13], MD is not

widely implemented. Owing to the risk of recurrence and the possibility of MD failure, surgical orchiopexy is recommended in patients after MD. These data might be the underlying reason for use of MD rarely in the clinical practice. However, MD may be important for testicular viability, especially in patients who has not undergone the operation immediately [13]. Is it possible to prevent intermittent torsion without the extra-scrotal method after MD? If this is the case, then this method may be completed with this non-invasive maneuver. Furthermore, this method would not only be minimally invasive, but also the general anes-

thetia risks during scrotal exploration would be prevented [3]. In this animal model, by testing the extra-scrotal fixation technique along with MD after CDU, we aimed evaluate the results of testicular fixation following MD to offer a new approach for TT therapy.

MATERIAL AND METHODS

Study design

The applicability of this new technique was evaluated with the present pilot study. The experimental protocol was reviewed and approved by the Local Animal Research Committee.

Animal subjects and handling

This study is a prospective, randomized, single-blinded, controlled trial in an animal model. Twelve New Zealand white male rabbits with similar ages and weights (range: 2.5–3 kg) were included in this study. They were taken to the surgery unit 7 days before the

beginning of the study. The main purpose of the study was to generate as much as different models of torsion and to evaluate their results rather than comparing the torsion models to each others. Therefore, based on a previous study [14] different degrees from 180° to 1080°, side and duration time of TT were assigned and the rabbits were randomly divided into six experimental groups: Group 1 (180°: 4 h), Group 2 (720°: 6 h), Group 3 (1080°: 9 h), Group 4 (540°: 1 h), Group 5 (900°: 2 h), and Group 6 (sham-only scrotal exploration). All animals were maintained at room temperature ($22 \pm 2^\circ\text{C}$) in a controlled 12/12-hour light/dark cycle. Standard laboratory feed and water were given during the study.

Torsion model

All of the animals were anesthetized intramuscularly via xylazine (5 mg/kg) and ketamine hydrochloride (30 mg/kg). The testicle was reached through a 2-cm inguinoscrotal vertical incision. For the groups, the testicle torsion model was implemented

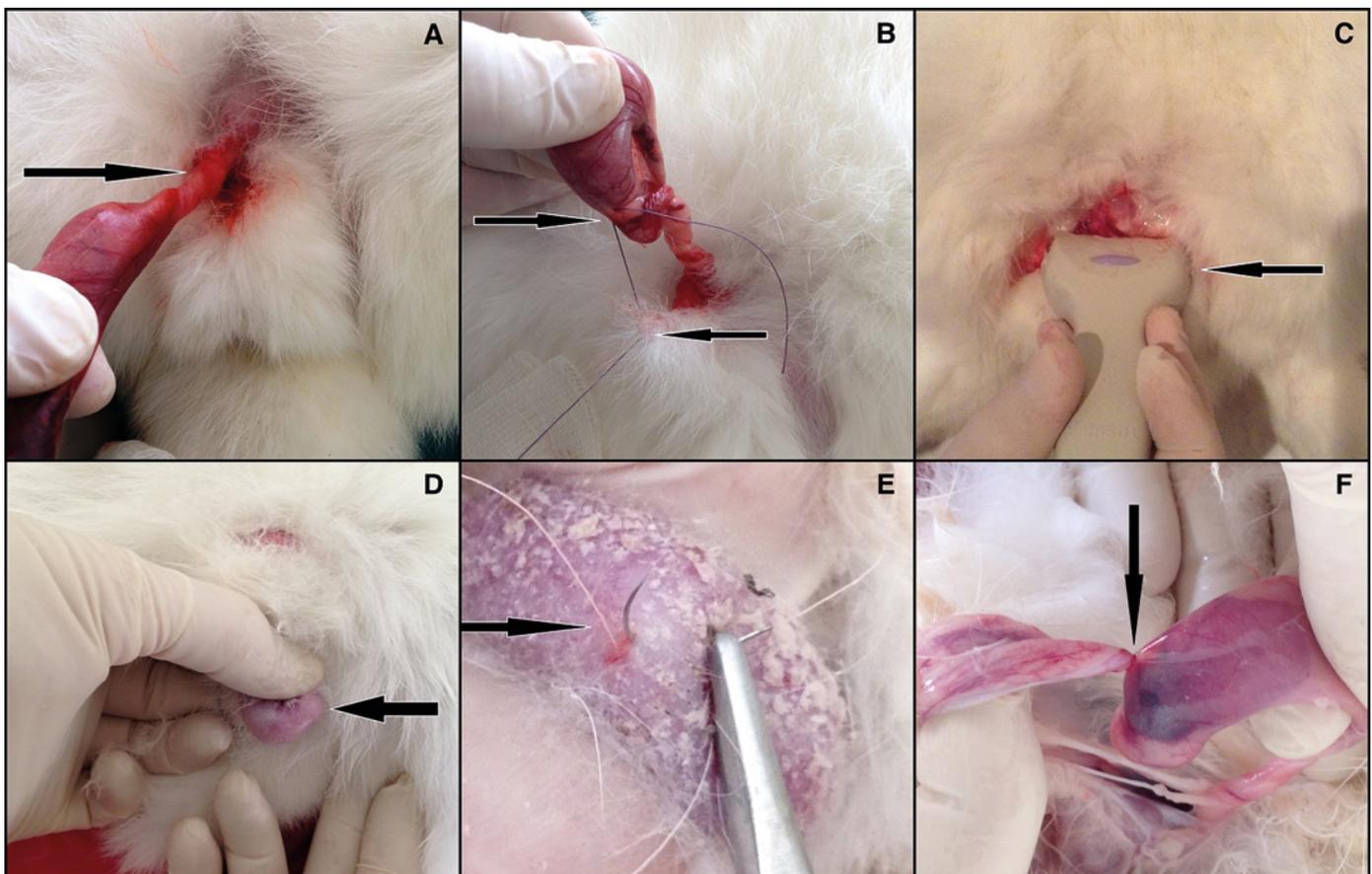


Figure 1. Steps of the surgical technique. (A) TT model in the counter-clockwise direction in the right testicle after an inguinoscrotal incision. (B) Fixing the twisted testicle within the scrotum in a way that left the node out of the skin. (C, D) Execution of MD along with single blind CDU via the Linear B mode probe. (E) Extra-scrotal fixation in the right testicle via MD allowing for blood flow. (F) Assessment of extra-scrotal fixation sutures between the tunica albuginea and scrotal wall in scrotal exploration.

in different directions (inward-outward), at different angles (180°–1080°) and for different durations (1–9 h) (Figure 1, part A). The 4/0 polyglactin fixation sutures passing through the tunica albuginea were fixed in a way that allowed the nodes to stay out of the scrotum. The skin incision was then closed (Figure 1B). This entire step was performed by one urologist.

Color Doppler Ultrasonography and Manual Detorsion

At the end of the duration period for each group a bedside B-mode ultrasound and CDU were performed by a radiologist by using a 12-5 MHz linear array transducer (Xario 200; Toshiba, Tokyo, Japan). A consultant radiologist and another urologist (second) attended the consultation. By the use of the CDU, the testicular blood flow, the presence of peritesticular liquid, spermatic cord diameter, testicular echogenicity and the twist direction (clockwise or counter-clockwise) were recorded (Figure 2A). A craniocaudal examination of the spermatic cord with CDU was performed to show the direction of torsion. After finishing the examination, by opening the fixation sutures previously created, MD was performed by observing the testicular blood flow (Figure 1, parts C and D). Spectral analysis of the central artery was confirmed by CDU following MD (Figure 2B).

Extra-scrotal fixation

The testes were manually fixed at the point where the arterial flow within the testicles was restored. With the other hand, an extra-scrotal entrance was executed through a 4/0 polyglactin surgical suture. By passing through the tunica albuginea, the needle was removed from the scrotum at another point. They were fixed to the scrotal wall at two points via surgical nodes at the inlet and outlet points (Figure 1E). At the end of the extra-scrotal fixation, scrotal exploration was performed on all rabbits under anesthesia. We examined the relationship between the tunica albuginea and scrotal wall, and residual rotation of the spermatic cord (Figure 1F). After the confirmation of extra-scrotal fixation, orchietomy was performed about 1 hour later and the testis samples were obtained for histopathological examination. The MD, extra-scrotal fixation and orchietomy steps were performed by the second urologist who was blind to the rotation degree, side and duration of TT. Radiologists also were blind to this data.

Histological procedures

The testicular samples were fixed in 10% buffered formalin and routinely processed for histopathologi-

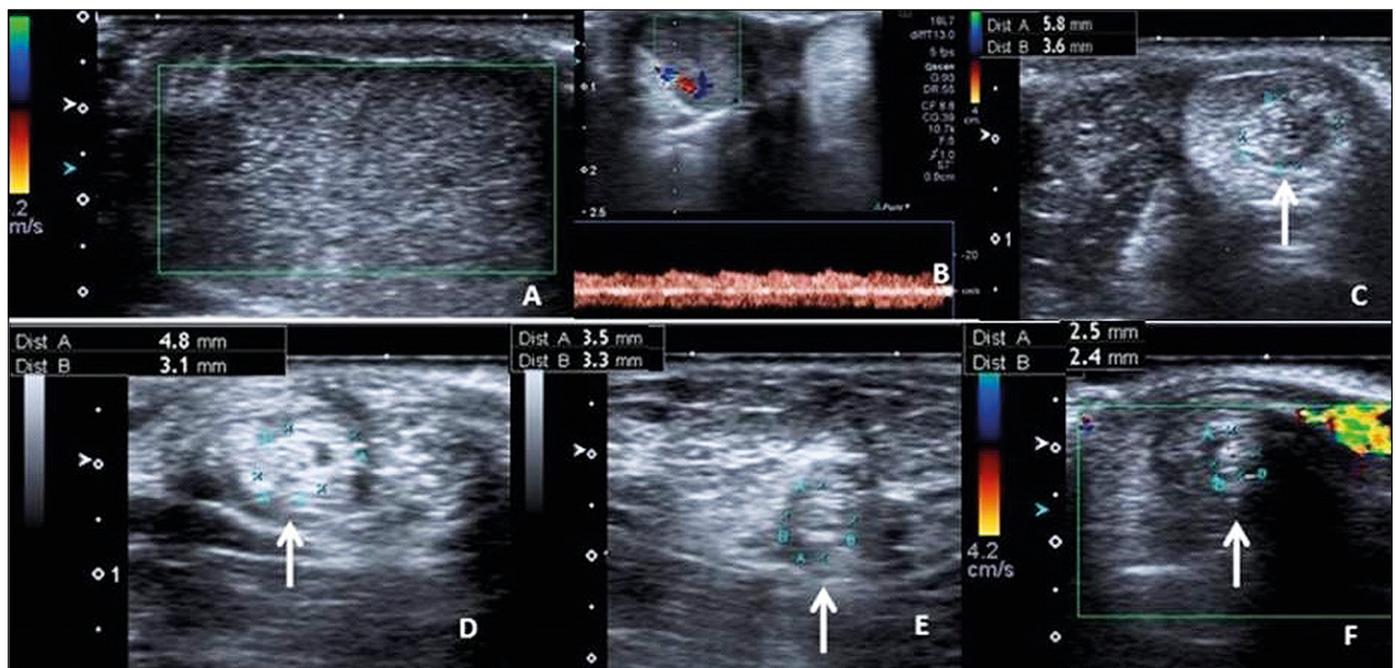


Figure 2. CDU evaluations of the testicles and spermatic cord during the procedures. (A) The longitudinal image of the torsive right testis shows no blood flow by color Doppler examination. (B) Spectral analysis of the central artery by CDU following MD shows the immediate occurrence of central perfusion. (C) The diameter of the neutral spermatic cord in the Sham group, where no torsion was observed. (D, E and F) Spermatic cord diameters decreasing as the torsion degree increases in Groups 1, 2 and 3. A: Transverse diameter; B: Antero-posterior diameter.

cal evaluation by embedding them in paraffin wax. Sections were cut in 5- μ m thicknesses and stained with hematoxylin and eosin (HE). The entire testicular histology was assessed blindly by two different pathologists. Light microscopy (Olympus CX41; Olympus Corporation, Tokyo, Japan) was utilized for the evaluation. Morphometric evaluations were performed by using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan). Testicular damage was evaluated and compared according to the duration of torsion in all groups.

Johnsen's tubular biopsy score (JTBS) was used for the semi-quantitative evaluation of spermatogenesis in 20 seminiferous tubules from each testicular section.¹⁵ In each of the groups, testicular tubule sections were classified by degrees, ranging from 1 to 10. In this classification, the value '10' indicates complete spermatogenesis and regular structures; a '9' indicates many spermatozoa present and disorganized tubules; an '8' indicates the presence of only a few spermatozoa; a '7' indicates no spermatozoa, but many spermatids are present; a '6' indicates no spermatozoa and only a few spermatids are present; a '5' indicates no spermatozoa or spermatids, but many spermatocytes are present; a '4' indicates the presence of a few spermatocytes; a '3' indicates the presence only spermatogonia; a '2' indicates no germ cells, but only Sertoli cells; and a '1' indicates the complete absence of germ cells and spermatogenesis.

RESULTS

All rabbits survived without major complications throughout the experiment. In groups 1 to 5, testicular torsions were completely observed. There was no torsion in the Sham group. In CDU, while decreased blood circulation was observed in Group 1, there was no blood circulation seen in the other torsion groups (Figure 2A). Spermatid diameters were inversely proportional to the degree of torsion (Figure 2C–F; Table 1). The residual degree of the torsion was measured as 180° in Group 3 and 90° in Group 5. The twisted direction of torsion was found in only Group 3 in CDU. A wrong direction of MD in the first step was observed in two rabbits in Groups 4 and 5.

Histopathological scores of the groups are given in Table 1. At the histopathological examination, while severe hyperemia and slight hemorrhage were observed in Group 1, no necrosis was detected. Spermatozoa, spermatogonia and Sertoli cells were normal in appearance and very slight neutrophil leukocyte infiltrations were observed (Figure 3A). In Group 2, marked hemorrhages and moderate ne-

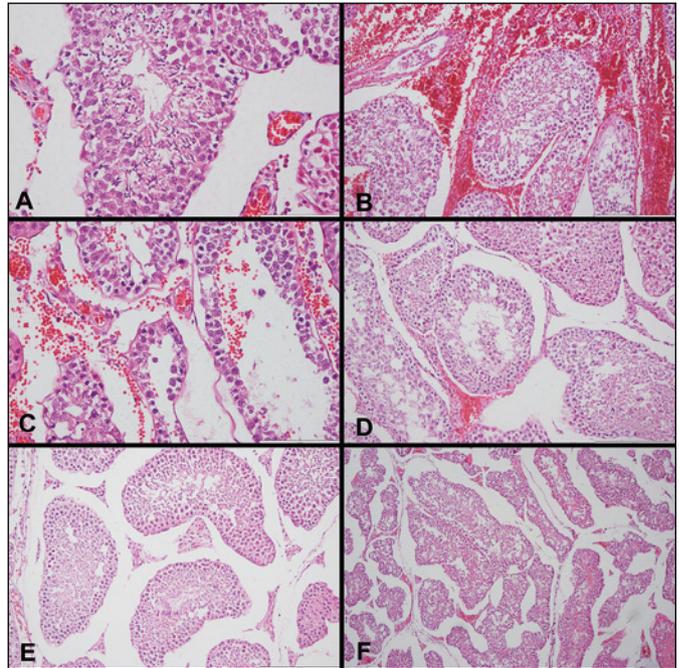


Figure 3. Histopathological evaluation of the testes. (A) In Group 1, there was slight hyperemia (thin arrows), normal tubulus seminiferus contortus and numerous spermatozoa in the tubular lumen (thick arrows). Bar: 100 μ m (B) In Group 2, there was a severe hemorrhage at the interstitial tissue (thin arrows), mild degeneration at spermatogonia (thick arrows) and Sertoli cells marked with necrosis (arrow head) in spermatozoa in the tubular lumen. Bar: 200 μ m (C) In Group 3, there was hyperemia and hemorrhage at the interstitial tissue with marked degeneration and a complete loss of the mature spermatozoa (thick arrows), but the basal membranes were intact. Bar: 100 μ m (D) In Group 4, there was a slight hemorrhage (thin arrows), spermatozoa in the lumen were still visible, spermatocytes, spermatogonia, Sertoli cells and basal membranes were intact. Bar: 200 μ m (E) In Group 5, the spermatocytes, spermatogonia (thick arrow), Sertoli cells and basal membranes were intact with no inflammatory reactions and tubulus seminiferous contortus were generally full with spermatozoa. Bar: 200 μ m (F) In Group 6, there was a normal appearance of the control testicles, tubular lumens completely full of spermatozoa (thick arrows) and no pathological findings in any cells. Bar: 400 μ m; H&E.

crosis were diagnosed. Slight degeneration was seen in the spermatogonia and Sertoli cells, while mild necrosis was observed in spermatozoa and spermatids. A slight inflammatory reaction was also observed in Group 2 (Figure 3B). In Group 3, while the tubular basal membranes were intact, marked necrosis was observed in spermatozoa, spermatids, spermatogonia and most of the Sertoli cells (Figure 3C). In Groups 4 and 5, spermatozoa were observed in only a limited number of tubules, but spermatocytes, spermatogonia, Sertoli cells and basal mem-

Table 1. Study design and results

	Group 1 n = 2	Group 2 n = 2	Group 3 n = 2	Group 4 n = 2	Group 5 n = 2	Sham n = 2
Degree of TT	180°	720°	1080°	540°	900°	0°
Duration of TT (Hours)	4	6	9	1	2	8
Direction of TT	clockwise	clockwise	counter-clockwise	counter-clockwise	clockwise	–
Side of affected testis	Right	Right	Left	Right	Left	Right-Left
Conformation of TT with CDU	-	-	+	-	-	-
Arteriel blood-flow velocity with CDU	decreased	absent	absent	absent	absent	normal
Diameters of SC (TV *AP) mm	4.8*3.1 5.0*3.7	3.5*3.3 3.5*3.1	2.5*2.4 2.3*2.2	4.0*3.2 4.0*3.4	2.9*2.6 3.1*2.5	5.8*3.6 5.7*3.5
The residue degree of the TT	–	–	180° (n=1)	–	90° (n=1)	–
JTBS score	8	5	3	9	9	10

TT; Testicular torsion, SC; spermatic cord, JTBS; Johnsen's testicular biopsy score, CDU; colour Doppler ultrasonography, AP; Antero-posterior, TV; Transverse, mm; millimeter

branes had a normal appearance. Although hyperemia and slight hemorrhages were observed, no inflammatory reactions were seen (Figure 3D and E). A normal testicle seminiferous tubule morphology was observed in the Sham group (Figure 3F).

DISCUSSION

Since the degree and direction of testicle's rotation in TT case were not known, MD may be accepted as a blind process in clinical practice. However, immediately upon resolution of scrotal pain and restoration of blood flow after MD, there was greater confidence in performing this procedure [7, 8, 16]. Many successful MD procedures have been reported in the literature [7–13]. Knowing the direction of torsion before the MD may ease the implementation of this procedure. While the direction may be determined in certain cases [13], spermatic cord assessments are often overlooked or ignored since the radiologist and urologist focus on scrotal exploration.

Although the testes rotate generally inwards, a significant amount of outward rotation has also been reported [14]. The first choice for many MD implementations in the literature is an outward derotation [7–12]. However, in atypical torsions, this selection may result in an increased torsion [17]. In our study, the torsion direction could be observed in only Group 3. This may be because the fine cord structures of rabbits are more difficult to observe than those of humans. In our study, all of the torsion groups were determined via CDU, and arterial flow restoration was observed in all animals following MD and extra-scrotal fixation. In contrast with the general direction of rotation, the first preference in MD was clockwise in the left testicle and counter-clockwise in right testicle. Thus, during MD implementation in two groups (Groups 4 and 5), detorsion was started in the wrong direction for four rabbits. Since no flow was observed in the CDU, detorsion was continued in the reverse direction until a flow was observed in the CDU. Because

the torsion direction could be predicted before MD only in Group 3, this error is considered inevitable. Moreover, the execution of MD under general anesthesia in this study might have contributed to the occurrence of this error because a sudden removal of the pain in humans was accepted as the proof of performing MD in the correct direction. Therefore, general anesthesia has not been used during this procedure [7, 8, 10–13].

One of the important results of this study was the inverse proportion between the degree of torsion and the cord diameter. The decrease in cord diameter due to an increased torsion degree may be an important finding for pre-MD in clinical practice. To the best of our knowledge, this finding has not been sufficiently discussed in the TT literature. This may be because this topic has been overlooked since the priority in TT cases is scrotal exploration. Clinically, this finding may contribute to the practice through its prediction that MD may be done better with finer measurements of the spermatic cord.

An important disadvantage of this technique is the residual torsion that may occur. In two rabbits in Groups 3 and 5, although arterial flow was observed in the spectral examination after MD, 180° and 90° residual torsions were observed. The presence of high torsion degrees in these groups may have played a role in this result. However, the exact reason for this is the insufficient execution of MD with a failure to ensure a neutral position of the spermatic cord.

Torsion duration is of vital importance in saving the testicle because durations exceeding 4–6 hours may result in testicular necrosis [18]. Depending on the pain duration, pathological changes may vary from congestion in the early period to hemorrhagic infarction in the late period [19]. It has been reported that irreversible ischemic changes occur at the 6th hour of scrotal pain [20]. In our study, in parallel with the literature, while no necrosis was observed in 4th hour and earlier (Groups 1, 4 and 5), irreversible changes were observed at the 6th hour (Group 2).

Again at the 9th hour (Group 3), there was significant necrosis observed. These histopathological findings corroborate the findings that the interventions that are made after the 6th hour may be less useful for saving the testicles [8, 14].

In actual approaches, even if the MD is successfully performed in TT, scrotal exploration and testicular fixation are recommended for preventing intermittent torsions. Although many methods have been described for this purpose, many surgeons use one or more absorbable or non-absorbable sutures through the tunica albuginea pathway [21–24]. We preferred using polyglactin sutures. Thus, the testicles were fixed at two points out of the scrotum through the tunica albuginea. Here, to the best of our knowledge, this technique has been described for the first time. The main limitation of this animal study was the use of a relatively low number of experimental rabbits. Another limitation is that we did not wait long

enough for the formation of adhesive tissue that may form between the tunica albuginea and scrotal wall. Furthermore, the potential effects of this method on fertility were not examined. Another important limitation is that all of the procedures were performed under general anesthesia. This is a limitation because, in humans, the pain response during MD is an indicator that the maneuver is in the appropriate direction [7, 8, 10–13].

CONCLUSIONS

Extra-scrotal fixation after MD along with CDU may be a simple and minimally invasive treatment option in TT therapy. However, this method should be confirmed with further – larger studies.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

References

- Aydogdu O, Burgu B, Gocun PU, et al. Near infrared spectroscopy to diagnose experimental testicular torsion: comparison with Doppler ultrasound and immunohistochemical correlation of tissue oxygenation and viability. *J Urol.* 2012; 187: 744-750.
- Nussbaum Blask AR, Rushton HG. Sonographic appearance of the epididymis in pediatric testicular torsion. *AJR Am J Roentgenol.* 2006; 187: 1627-1635.
- Boettcher M, Krebs T, Bergholz R, Wenke K, Aronson D, Reinshagen K. Clinical and sonographic features predict testicular torsion in children: a prospective study. *BJU Int.* 2013; 112: 1201-1206.
- Kalfa N, Veyrac C, Baud C, Couture A, Averous M, Galifer RB. Ultrasonography of the spermatic cord in children with testicular torsion: impact on the surgical strategy. *J Urol.* 2004; 172: 1692-1695.
- Güneş M, Umul M, Altok M, et al. Is it possible to distinguish testicular torsion from other causes of acute scrotum in patients who underwent scrotal exploration? A multi-center clinical trial. *Cent European J Urol.* 2015; 68: 252-256.
- Eaton SH, Cendron MA, Estrada CR, et al. Intermittent testicular torsion: diagnostic features and management outcomes. *J Urol.* 2005; 174: 1532-1535.
- Garel L, Dubois J, Azzie G, Filiatrault D, Grignon A, Yazbeck S. Preoperative manual detorsion of the spermatic cord with Doppler ultrasound monitoring in patients with intravaginal acute testicular torsion. *Pediatr Radiol.* 2000; 30: 41-44.
- Cornel EB, Karthaus HF. Manual derotation of the twisted spermatic cord. *BJU Int.* 1999; 83: 672-674.
- Harvey M, Chanwai G, Cave G. Manual testicular de-torsion under propofol sedation. *Case Rep Med.* 2009; 529346.
- Townsend RR. Color Doppler Evaluation of Testicular Torsion with subsequent Blood Flow After Immediate Manual Detorsion. *J Diagn Med Sonogr.* 1999; 15: 197-202.
- Wang S, Scoutt L. Testicular torsion and manual detorsion. *Ultrasound Q.* 2013; 29: 261-262.
- Bomann JS, Moore C. Bedside ultrasound of a painful testicle: before and after manual detorsion by an emergency physician. *Acad Emerg Med.* 2009; 16: 366.
- Güneş M, Umul M, Çelik O, Armağan HH, Değirmenci B. A novel approach for manual de-torsion of an atypical (outward) testicular torsion with bedside Doppler ultrasonography guidance. *Can Urol Assoc J.* 2015; 9: 676-678.
- Sessions AE, Rabinowitz R, Hulbert WC, Goldstein MM, Mevorach RA. Testicular torsion: direction, degree, duration and disinformation. *J Urol.* 2003; 169: 663-665.
- Johnsen SG. Testicular biopsy score count—a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormones.* 1970; 1: 2-25.
- Aminu S, Syed K, Gunendran T, Young G. A fusional anomaly of the epididymis associated with recurrent testicular torsion. *Cent European J Urol.* 2012; 65: 51-52.
- Güneş M, Umul M, Altok M. Atypical testicular torsion: we must be cautious in the course of manual de-torsion. *Ped Emerg Care.* 2016; 32: 691-692.
- Mansbach JM, Forbes P, Peters C. Testicular torsion and risk factors for orchiectomy. *Arch Pediatr Adolesc Med.* 2005; 159: 1167-1171.
- Visser AJ, Heyns CF. Testicular function after torsion of the spermatic cord. *BJU Int.* 2003; 92: 200-203.
- Kapoor S. Testicular torsion: a race against time. *Int J Clin Pract.* 2008; 62: 821-827.
- Pul M, Pul N, Yildiz K, et al. Comparison of the effects of surgical and autologous fascial suture materials on testicular histology in ratssubjected to orchiopexy. *Int Urol Nephrol.* 1991; 23: 169-175.
- Steinbecker KM, Teague JL, Wiltfong DB, Wakefield MR. Testicular histology after transparenchymal fixationusing polytetrafluoroethylene suture: an animal model. *J Pediatr Surg.* 1999; 34: 1822-1825.
- Gesino A, Bachmann De Santos ME. Spermatic cord torsion after testicular fixation. A different surgical approach and a revision of current techniques. *Eur J Pediatr Surg.* 2001; 11: 404-410.
- Antao B, MacKinnon AE. Axial fixation of testes for prevention of recurrent testicular torsion. *Surgeon* 2006; 4: 20-21. ■