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Research article
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Ultrasonographic findings of intrascrotal testicular torsion at the early stage in a rabbit model

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ABSTRACT: Intrascrotal testicular torsion (ISTT) cases are rarely seen in companion animals, harming both testicular functions. These are considered reproductive emergency cases and need acute surgical intervention. In this research, early scrotal ultrasonographic findings were assessed in a rabbit model. Unilaterally, complete clockwise torsion was inducted in the left testicles of eight healthy pubertal male rabbits. The right testicle of each animal was considered as control. B-mode and Doppler ultrasonography (USG) was performed preoperatively (-1st) and postoperatively (2,4,6, and 24th) hours (h). At the first two hours, swelling, cyanosis, hypothermia, and pain symptoms were observed in all rabbits (100%). Reactive hydrocele was sonographically detected at 2-h (12.5%; 1/8), 4-6, and 24-h (50%; 4/8), respectively. At 2nd h, hyperechogenic whirlpool sign was remarkable in transversal and sagittal scans of the spermatic cord in all cases (100%). At 6th h, increased echogenicity of the spermatic cord and testicular parenchyma were also observed in all torsed testes (100%). Except for the scrotal capillary vessels in the distal part of torsion, testicular and epididymal blood flow were absent in all rabbits (100%). Increased pulsatility index (PI) at the second h and decreased resistance index (RI) at the 4th and 6th h in the control group ($P<0.05$) was observed. At the end of the study, RI was increased at 24th h in both groups ($P<0.05$). The mild hydrocele and whirlpool signs are clear and remarkable diagnostic findings of ISTT cases in rabbits. By twisting at the spermatic cord, increased echogenicity and absence of blood flow from the twisted area to the distal part of the spermatic cord help the differential diagnosis. The torsion causes the increasing RI in contralateral testicular perfusion after 24 hours of the event. In conclusion, testicular echogenicity, whirlpool signs, visibility of the blood flow, and its spectral waveform features are useful measurements in diagnosing ISTT cases. Moreover, the time of torsion and prognosis may also be estimated.

***Keywords*:** Testicular torsion, ultrasonography, whirlpool, rabbit model.

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INTRODUCTION

Intrascrotal testicular torsion (ISTT) is causedby self-torsion of the testes and is a urological emergency in infants and young males, requiring acute surgical intervention to avoid ischemia necrosis (Schneck and Bellinger, 2007; Kapoor, 2008). In dogs, spermatic cord torsion is a rare condition and can be related to cryptorchidism and concomitant testis neoplasia (Hulse, 1973; Pearson and Kelly, 1975; Young, 1979; Laing et al., 1983; Mibayashi et al., 1990; Feldman and Nelson, 1996; Quartuccio et al., 2012). In many reports, mostly abdominal pain, the cases have appeared into cryptorchidic young male dogs with abdominal pain, skin alopecia, weight loss (Pearson and Kelly, 1975; Mibayashi et al., 1990; Hecht et al., 2004; Quartuccio et al., 2012; Carr et al. 2015 Mostachio et al., 2007; Howser and Vinayak, 2018). Intrascrotal cases are much more rarely seen than intraabdominal ones thatare often reported in canine and feline literature (Zymet, 1975; Young, 1979; Crivellenti et al., 2012 Giuliano, 2013; Villiotti et al., 2018). Tunica vaginalis of the descended testicle does not sufficiently support conjointly to the scrotum (Guerra et al., 2008; Djahangirian et al., 2010). Although this rare pathology is described as an idiopathic condition regardless of age and race; trauma, excessive physical activity, and rupture of the scrotal ligament are associated as etiologic factors in normally descended testicles (Melikoğlu et al., 1992; Hoşcan et al. 2012; Villiotti et al., 2018).

This acute and emergent disease causes sudden/great pain with scrotal edema, subsequent necrosis of the gonad (Feldman and Nelson, 1996), and contralateral organ damage. They are thus releasing acrosome enzymes, neuroendocrine/vasomotor responses, and dramatic perfusion changes (Melikoğlu et al., 1992; Hoşcan et al., 2012). Therefore, quick and definitive clinical diagnosis plays a vital role in the surgical procedure pathway, up to the necropsy report. Ultrasonography (USG) is a routineexam to diagnose and differentiate testicular torsion from other pathologies with similar symptoms. Increased echogenicity and absent blood flow in the distal part of torsed tissue describe the torsion cases (Pinto et al., 2001; Bartlett, 2002; Hecht et al., 2004). Besides these signs, the remarkable changes in the spermatic cord's form like that whirlpool, snail shell, target, storm are very definitive in acute ISTT cases. This change is reported as the most specific and sensitive sign on Grayscale (Vijayaraghavan 2006). However, no reports about this finding in the testicular cases' veterinary literatureare available, except for some mesenteric disorders (Spekabow et al., 2010; Arronson, 2016). In the light of earlier literature, this experimental study aimed to evaluate the B-mode and Doppler scans' imaging features during the first 24 hours of acute testicular torsion for pet animals' diagnostic purposes.

MATERIAL AND METHODS

This study was conducted with ethical approval of Adnan Menderes University, Local Ethics Committee of Animal Experiments (ADU-HADYEK), 64583101/2017/018. During this study, eight five-month-old male New Zealand healthy rabbits were used. All the animals were fed ad libitum in individual wire-mesh cages under controlled conditions of heat (18-24 °C) and light (14 h light, ten h dark).

The left testicles were defined as the experimental side in all the animals. Animals were anesthetized with an intramuscular injection of 35 mg/kg ketamine (Alfamine %10 - Ege Vet®), and 5 mg/kg xylazine (Alfazyne %2 - Ege Vet®). Following aseptic preparation, the left testis was delivered via a longitudinal scrotal skin incision. Testicles were twisted 360° in a complete clockwise direction and sutured by 3/0 vicryl sutures to the scrotum, according to the experimental procedure reported in previous studies (Acar, 2005; Hoşcan, 2012). Right testicles without any application were served as an internal control.

During the torsion procedure, B-mode and Pulsed wave Doppler USG of testicles was performed with an 8.0 MHz microconvex probe (Mylab 30-Esaote®, Genova, Italy) preoperatively (-1^{sth}h) and postoperatively (2, 4, 6, and 24thh). In B-mode USG, longitudinal and transverse scans of testicles were obtained to detect acute changes in spermatic cord and testicles (increased echogenicity, hydrocele, whirlpool sign reflected its tortuosity) depending on the duration of the event. After B-mode scans, Color and Pulsed Doppler USG were performed to evaluate the vascular supply of the gonads. Pulsatility index (PI) and resistance index (RI) measurements were recorded in spectral traces of the arteria testicularis in the proximal part of the twisted area of test groups and symmetrical regions control group. All measurements were generated using the manual mode after obtaining a minimum of three consecutive convenient artery wave images.

The data were evaluated using SPSS 22.0 (SPSS Inc., Chicago, IL, USA) package program. In comparing the data in Group 1 (Twisted) and Group 2 (Control), the normally distributed data were evaluated by paired t-test. To determine the group's time-dependent changes, the data were assessed by repeated two-way analysis of variance. The data in the table, graphics, and results section are expressed as mean ± standard deviation. Statistical significance was accepted as $p < 0.05$.

RESULTS

At the first two hours of the induction, it was observed that the left testes were localized on the proximal part of the scrotum, and the swelling, cyanosis, hypothermia, and pain symptoms were observed in all rabbits (100%).

Reactive hydrocele findings were detected as an anechogenic area surrounding testicles without particles reflecting hyperechoic brightness. These findings were recorded at 2nd h examination as 12.5% (1/8); 50% (4/8) at 4-6, and 24th h USG exams, respectively (Figure 1). The increase of the echogenicity in the left spermatic cord and torsed testicular parenchyma was remarkable and easily visible. Moreover, the absence of blood flow was in the torsed left testis at bilateral comparatively scans (Figure 2).

In all rabbits (100%), a hyperechogenic whirlpool sign was seen in transverse scans (Figure 3). Accordingly, twist signs in longitudinal scans of the spermatic cord after 2-h (Figure 4). Especially in longitudinal scans, hyperechogenicity of the distal part of the spermatic cord (D) was prominent when comparing the proximal portion (Figure 4). At 6th h, increased echogenicity of the spermatic cord and testicular parenchyma was also observed in all torsed testes (100%). Except for the scrotal capillary vessels in the distal part of torsion, during Doppler scans in both dimensions, there is the absent blood flow of testicular and epididymis parenchyma.

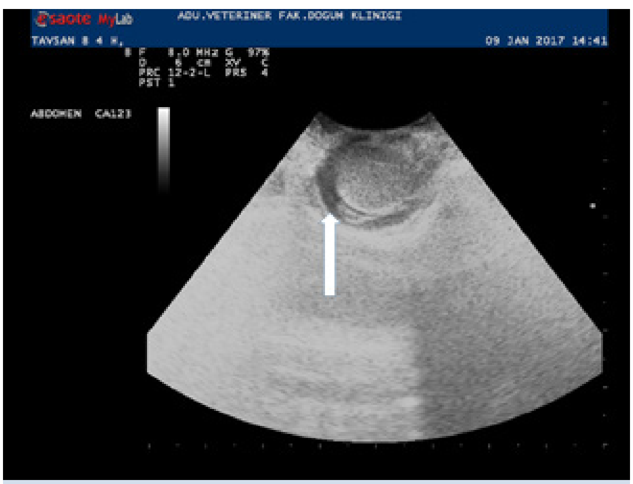

Figure 1: Mild reactive hydrocele at torsion testicle

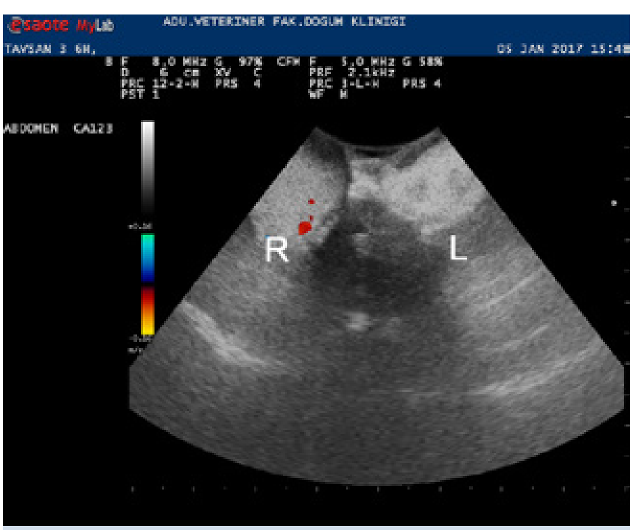

Figure 2: Increased echogenicity and absent flow in the left testicle (L) comparing with right testicle (R)

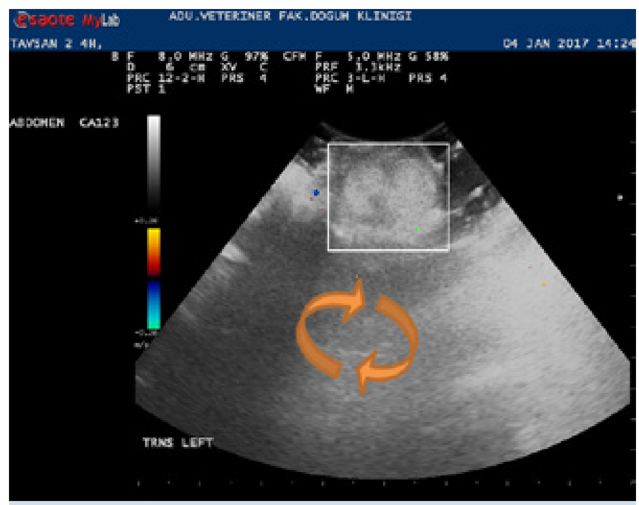

Figure 3: Whirlpool sign - transversal scan

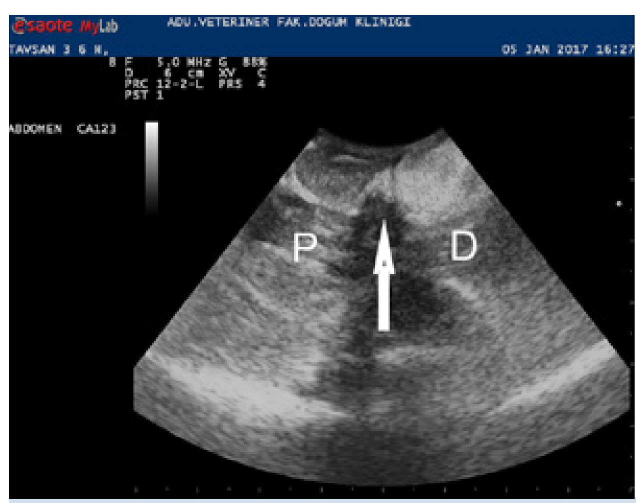

Figure 4: Twisting sign (arrow), absent flow, and increased echogenicity in the distal part (D) comparing with the proximal part of torsion (P) - at longitudinal scan

During the spectral Doppler examination, there was no asymmetry, reverse of diastolic flow, or other possible pathologic findings in traces. The mean of the PI and RI of the blood flow of arteria testicularis in groups were presented in Table 1. There was no significant difference between the groups than at the same time exams (P>0.05). However, when related to the time-dependent changes in the groups, PI values were higher at the 2nd h in the control group (Figure 5) (P<0.05). Resistance index was lower at the 4th and 6th in the control group (P<0.05), but also increased at 24th h in both groups (Figure 6) (P<0.05).

Following the last examinations, a castration operation was done in all animals under general anesthesia. Macroscopic findings revealed that the enlarged left testicles had blackened coloration and generalized hemorrhage in all torsed testicular necropsy material (100%).

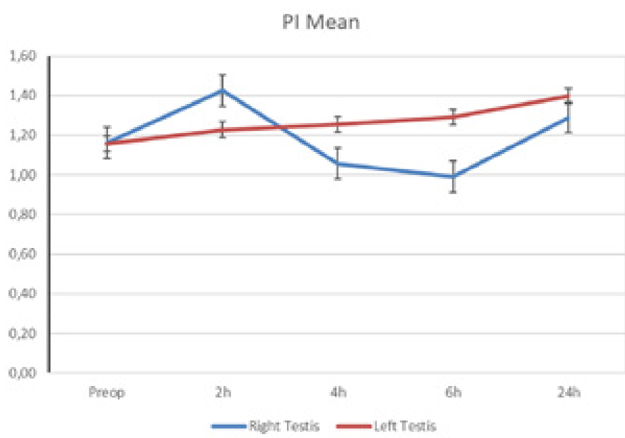

Figure 5: The mean PI of both study groups

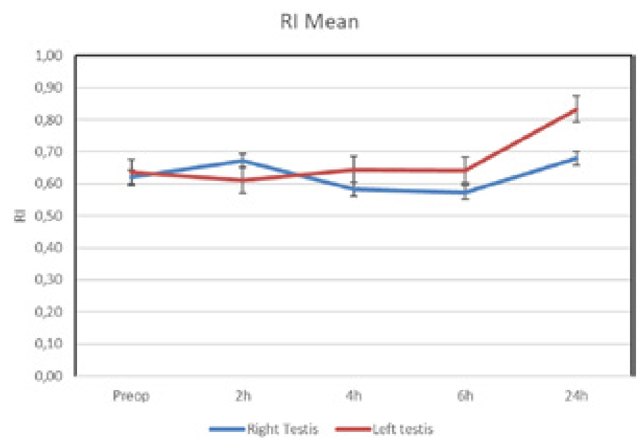

Figure 6: The mean RI of both study groups

Table 1: The mean of the PI and RI of the blood flow of arteria testicularis in study/control groups

Doppler parameters	Preoperatively	2 h	4 h	6 h	24 h
PI Torsion	1.15±0.09	1.22±0.09	1.25±0.10	1.29±0.14	1.39±0.13
PI Control	1.16±0.06	1.42±0.14*	1.05±0.10	0.99±0.11	1.28±0.09
RI Torsion	0.63±0.03	0.61±0.02	0.64±0.02	0.64±0.03	0.83±0.10*
RI Control	0.62±0.02	0.67±0.03	0.58±0.02*	0.57±0.03*	0.67±0.03*

Values are represented as mean ± SD. * P < 0.05.

DISCUSSION

The clinical and physical signs of testicular torsion can mimic those seen in cases of epididymo-orchitis (Berman et al., 1996). Therefore, different scrotal USG techniques may help differentiate acute intrascrotal pathologies (Vijayaraghavan, 2006; Cassar et al., 2008). Long-term ischemic condition in tissues results in irreversible defects on the male's reproductive capability. If the surgical detorsion of a complete torsed testis is not performed by 4-6 hours or more, infarction and necrosis of the gonads are inevitable (Harisinghani et al., 2019). Ferreira (2000) reported that the first 2 hours is the critical period of ischemia survival in canine germ cells. This studyproved the most specific findings of Grayscale and applicability of Spectral Doppler examinations in the diagnosis of ISTT on the rabbit model.

In ISTT cases, fluid collection in the scrotum by venous obstruction is the most common finding, which is more apparentafter 4 hours in Grayscale imaging (Sirivastava, 2017; Harisinghani et al., 2019). In the orchitis or other infectious condition in patients, this sign appears as a hematocele or pyocele (Berman et al., 1996). In our study, reactive hydrocele was one of the first seen complications in Grayscale caused by compression of the testicular blood flow. As the result of an aseptic experimental induction, an anechoic fluid accumulation surrounding the torsed testicles was seen in the scrotal sac. The amount of this fluid was variable and could not be measured. In our study, 4 hours after the torsion, the visibility rates of hydrocele sign increased to 50% from 12.5% previously reported in the literature. Besides, in half of the test group, hydrocele was still detected during 6, and

24^{th} h examinations. Regarding that, the first 4 hours are most critical for salvaging, and a mild-moderate hydrocele sign can be thought of as an alerting sign for "delayed case" for complete torsion cases.

Another specific data of torsion cases is the whirlpool sign in the twisted spermatic cord mass on Grayscale (Vijayaraghavan, 2006; Cassar, 2008). The flow in the whirlpool mass vessels helpsto differentiate incomplete torsion cases (Vijayaraghavan, 2006). In our study, a whirlpool sign was recorded in all animals immediately after induction. As time goes by, the half part of the whirlpool got brighter (Figure-4). Moreover, absent flow and increased echogenicity were in the distal portion of the spermatic cord detected. By evaluating the whirlpool sign's sagittal scans, the torsion's direction could be detectable in some rabbits (Figure 4). In the evaluation of vascularity, the absence of blood flow in testis brings out more than 1800 torsion (Mevorach et al., 1991; Berman et al., 1996; Vijayaraghavan, 2006; Cassar, 2008; Howser and Vinayak, 2018; Villioti et al., 2018). As previously reported in the literature, we also observed the absence of blood flow toward the torsed testicle in the presented study. Regarding our observations, practitioners should be able to differentiate the capillary flow on the scrotal wall during Color Doppler scans.

The usefulness of spectral Doppler USG has been proven in previous studies (Middleton et al., 1989; Lerner et al., 1990). Testes with partial torsion can cause variable spectral patterns (increased, similar, or decreased amplitude) of the intratesticular arterial waveform relative to the contralateral testicle (Cassar et al., 2008). Nevertheless, the changes of the intratesticular and spermatic arterial waveforms are not in the veterinary literature up to the authors' knowledge. In the present study, spectral Doppler examination of the intratesticular blood flow was impossible in the test group because of the experimentally induced complete torsion in our rabbit model. Nevertheless, it was able to see the significant changes in the twisted spermatic cord's proximal part. These changes were noticed between the contralateral testes, related to the torsion hours' duration, contralateral. Acute response to torsion was detected as the high PI in the contralateral side at 2^{nd} h. On the torsion side, nonsignificantly, we recorded a linear increase in PI.

The mean RI is described as 0.62 (0.48 - 0.75) for healthy males, but this is not a reference value in diagnosis in partially torsion cases (Middleton et al., 1989). Although the high RI is considered suspicious for the partial testicular torsion, researchers pointed out that Pulsed Doppler analysis should be performed from the different parts of the testicles for proper diagnosis due to its subtle variations (Cassar et al., 2008). In this study, there was only one area (proximal part of the twisted portion) having the possibility of performing USG, and no difference was between the study groups. Regarding the RI variation in our study's results, the lower RI at $4^{th}$and 6^{th} h in the torsion group and high value at 24^{th}h in controls were similar to those described in the literature. These RI trends are hard to explain due to the rarity of reported literature of similar cases in the rabbit. Based on the referred healthy RI limits, although it is impossible to catch any testicular resistance difference during the first six hours,tissue damage can be detected sonographically with a high RI level 24^{th}h. In the subsequent studies, by performing the other exams between 6-24^{th}h, it can be possible to determinehigh testicular resistance threshold time.

CONCLUSION

In conclusion, the Gray- Color and Doppler USG scans are valuable tools in diagnosing the ISTT cases even in small-size pet animals. Testicular echogenicity, whirlpool signs, blood flow detection, and its spectral waveform features are useful measurements in this small animal practice's emergency case. Based on these parameters, the time of torsion and prognosis may also be estimated.

CONFLICT OF INTEREST

None declared.

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