Effect of Antioxidants on Outcome of Testicular Torsion in Rats of Different Ages

Carina T. Ribeiro, Raquel Milhomem, Diogo B. De Souza,* Waldemar S. Costa, Francisco J. B. Sampaio and Marco A. Pereira-Sampaio

From the Urogenital Research Unit, State University of Rio de Janeiro (CTR, RM, DBDS, WSC, FJBS, MAP-S), Rio de Janeiro and Department of Morphology, Fluminense Federal University (MAP-S), Niteroi, Brazil

Abbreviations and Acronyms

ARG = TT and arginine
ARG4 = 4-week-old ARG
ARG6 = 6-week-old ARG
ARG9 = 9-week-old ARG
Av = absolute volume
R = right testicle
RES = TT and resveratrol
RES4 = 4-week-old RES
RES6 = 6-week-old RES
RES9 = 9-week-old RES
SH = sham operation
SH4 = 4-week-old SH
SH6 = 6-week-old SH
SH9 = 9-week-old SH
TT = testicular torsion
TT4 = 4-week-old TT
TT6 = 6-week-old TT
TT9 = 9-week-old TT
Vv = volumetric density

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* Correspondence: Urogenital Research Unit, State University of Rio de Janeiro, Av. 28 de Setembro, 87-Fundos, Rio de Janeiro, RJ, Brazil, 20551-030 (telephone and FAX: + 55 21 2868-8399; e-mail: diogobenchimol@gmail.com).

Purpose: We assessed reproductive and testicular function in adult rats after testicular torsion created before, during and after puberty, and with vs without resveratrol or arginine treatment.

Materials and Methods: Age matched rats were divided into groups, including simulated surgery without testicular torsion, 720-degree testicular torsion for 4 hours, testicular torsion with resveratrol treatment and testicular torsion with arginine treatment. To study reproductive function at age 12 weeks each rat mated with 3 females. The males were sacrificed at age 14 weeks. Spermatozoids were collected from the epididymal tail and evaluated for concentration, motility and viability. Testicular samples were collected for morphological analysis.

Results: Reproductive function was not altered by testicular torsion but antioxidants improved potency. Compared to sham operated and contralateral samples all spermatozoid parameters from testicular torsion samples were inferior. Resveratrol and arginine did not improve spermatozoid quality or quantity in torsed testes but contralateral samples were improved by each drug. The seminiferous epithelium of rats submitted to testicular torsion during puberty was least affected. Each antioxidant partially to totally prevented the morphological alterations found in rats with untreated testicular torsion. Rats submitted to testicular torsion before puberty that were treated with antioxidants showed the fewest changes.

Conclusions: Testicular morphology was altered less in rats when torsion occurred earlier in life, that is during puberty. Treatment with antioxidants improved contralateral spermatozoid production and some fertility parameters. Each antioxidant also prevented testicular morphology alterations after testicular torsion. Prepubertal rats benefited most from antioxidant treatment.

Key Words: testis, spermatic cord torsion, puberty, resveratrol, arginine

TESTICULAR torsion is a urological emergency that induces biochemical and morphological changes.[1] TT can affect males of any age but it occurs more often in neonates, boys and young men.[2] To our knowledge the impact on prognosis of age at TT is unknown.

The prognosis of TT is related to the duration and degree of torsion, resulting in different levels of parenchymal injury by oxidative stress.[3] Therefore, beyond rapid diagnosis and treatment several methods have been investigated to minimize the injury caused by TT.[4,5] Although rat

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testes differ somewhat from human testes, rats have been widely used as experimental models in TT studies because lesions in rat testes are comparable to those in human testes after torsion.[6]

Several antioxidants have been investigated with promising results in rats submitted to TT. Of these antioxidants resveratrol and arginine have shown good results when used in testicular ischemia and reperfusion situations.[7,8] Arginine, an amino acid with antioxidant properties, is important for nitric oxide synthesis.[9] Resveratrol is a potent antioxidant present in many food sources that has inhibitory activity against reactive oxygen species and also enhances nitric oxide bioavailability.[10]

Although some groups reported beneficial results using these drugs, no quantitative evaluation of testicular parenchyma was performed. Also, to our knowledge no study has addressed morphological damage to the torsed testis, spermatozoid production or reproductive function after resveratrol and arginine treatment in rats submitted to TT at different ages.

We quantitatively assessed testicular morphology, spermatozoid parameters and reproductive function in adult rats that underwent TT before, during and after puberty. We also evaluated the effect of resveratrol and arginine treatments.

MATERIALS AND METHODS

We used 106 male Wistar rats for TT experiments. Three unrelated females per male (total 318) were used for the fertility tests described. The rats remained with the mother until week 3 of life. They were then kept in a room with a controlled temperature (mean $\pm$ SD 25C $\pm$ 1C) and artificial dark-light cycle (lights on from 7:00 am to 7:00 pm), and had free access to standard rat chow and water. All experiments were done by blinded observers according to the Brazilian law for scientific use of animals and they were approved by the local ethics committee.

Male rats were randomly assigned to 12 groups. The 3 age groups were prepuberty (4 weeks), puberty (6 weeks) and adulthood (9 weeks). The treatment groups were SH, TT without antioxidant therapy, RES and ARG. The SH group included 10 prepubertal, pubertal and adult rats each. The TT group included 10 prepubertal, 9 pubertal and 9 adult rats. The RES and ARG groups included 8 prepubertal, pubertal and adult rats each.

After general anesthesia induction TT was induced by opening the scrotum and the lamina parietalis of the tunica vaginalis, and twisting the right testis 720 degrees clockwise. The torsed testicle was fixed in position by sutures and torsion was maintained for 4 hours with the rat under general anesthesia. Rotation duration and degree were based on a previous study showing that they produced significant damage in the rat testis.[11] After this period the organs were untwisted and fixed in anatomical position. In SH rats the same surgical approach was used to open the tunica vaginalis. The testicle was sutured in anatomical position for the same period but not twisted.

RES4, RES6 and RES9 rats received resveratrol (30 mg/kg) intraperitoneally 30 minutes before testicle detorsion. For 7 days postoperatively resveratrol was administered daily by gavage at the same dose.[12] ARG4, ARG6 and ARG9 rats received arginine (650 mg/kg) by gavage for 7 days postoperatively.[13]

At age 12 weeks all male rats were mated with 3 estrous females to determine fertility parameters.[14] Females were sacrificed on day 20 of gestation. The uterus was opened, pregnancy was confirmed and the number of fetuses and implantation sites was recorded. The ovaries were observed under magnification and the number of corpora lutea was counted. Potency was calculated as the percent of female rats with confirmed copulation divided by the number exposed for mating. The fertility index was calculated as the percent of implantation sites divided by the number of corpora lutea. The fecundity of each group was considered the percent of male rats that generated at least 1 fetus divided by the total number of male rats in the same group. We also calculated preimplantation and postimplantation losses.

All males were sacrificed at age 14 weeks by anesthetic overdose. Just after sacrifice spermatozoids were collected from the epididymal tail to determine concentration and motility in a Neubauer chamber.[15] Spermatozoid viability was assessed by the hypo-osmotic test.[16] In this analysis 200 spermatozoids were evaluated per rat. Samples were collected and analyzed from the right torsed and the contralateral epididymides.

After sacrifice each testicle was dissected from the appendix and weighed. Volume was measured using the Scherle method.[17] The organ was then fixed and processed for paraffin embedding to obtain 5 μm histological sections. Morphometric analysis was performed on hematoxylin and eosin stained slices and captured on a BX51 microscope with a coupled DP70 digital camera (Olympus, Tokyo, Japan).

Testicular structure Vv was assessed by the point counting method.[17] Using ImageJ (http://rsb.info.nih.gov/ij/) we superimposed a test grid with 100 points over the testicular photomicrographs. Each structure touched by a point was counted and density was determined as a percent of the analyzed field.[18] For each testicle 25 fields were evaluated under 400× magnification. We recorded the Vv of the tunica propria, seminiferous epithelium, tubular lumen, seminiferous tubule (the sum of these 3 structures), the vessels and the intertubular compartment, including vessels.

We calculated the Av of each mentioned structure by dividing testicular volume by structure Vv, expressed in ml.[19] Total tubular length was calculated as previously described.[20]

The diameter of 125 seminiferous tubules per rat was measured in each testis by applying a straight line that crossed the tubule. For this purpose we used ImageJ, which was previously calibrated to 100× magnification. The line was applied in such a manner that it always passed through the center of the tubule. For this analysis we excluded tubules with an irregular shape.[21] Also, using this software the seminiferous epithelium height of randomly selected tubules was measured in each testis.

In this analysis 125 tubules per animal were assessed in images photographed at 200× magnification.

Cellular proliferation was evaluated separately in the interstitial and tubular spaces. Histological sections were immunostained with proliferation cell nuclear antigen antibodies (180110) with Histostain®-Plus labeling (859643, Invitrogen™). These sections were photographed under 400× magnification. The number of positive cells per mm^2 in the interstitium and seminiferous tubules was quantified using ImageJ.

We compared results in TT4, TT6 and TT9 rats to determine the possible role of age at TT onset in adult testicular function and morphology. Results in the SH, TT, RES and ARG groups were compared to verify whether antioxidant treatment could prevent testicular damage.

For each parameter results were initially analyzed by the Kolmogorov-Smirnov normality test. Parametric data were then compared by 1-way ANOVA with the Bonferroni post test. Nonparametric data were compared by the Kruskal-Wallis test with the Dunn post test. All analysis was done in Prism® 4.0. Mean differences were significant at $p < 0.05$. All results are shown as the mean ± SD.

Table 1. *Fertility test data by age at onset*

	Mean ± SD SH	Mean ± SD TT	Mean ± SD RES	Mean ± SD ARG
		Prepubertal		
Potency	0.61 ± 0.15	0.50 ± 0.23	0.76 ± 0.22*	0.83 ± 0.23*
Fertility	0.89 ± 0.15	0.77 ± 0.29	0.93 ± 0.09	0.95 ± 0.07
Fecundity	1.00 ± 0.00	0.90 ± 0.31	1.00 ± 0.00	1.00 ± 0.00
Losses:				
Preimplantation	0.10 ± 0.15	0.12 ± 0.06	0.06 ± 0.09	0.04 ± 0.07
Postimplantation	0.10 ± 0.14	0.05 ± 0.07	0.05 ± 0.04	0.05 ± 0.04
Fetuses/litter	11.0 ± 2.57	11.31 ± 1.62	11.83 ± 1.44	11.68 ± 1.28
		Pubertal		
Potency	0.56 ± 0.31	0.33 ± 0.28	0.80 ± 0.26*	0.95 ± 0.11*,†
Fertility	0.83 ± 0.12	0.85 ± 0.19	0.98 ± 0.07	0.89 ± 0.08
Fecundity	0.90 ± 0.31	0.66 ± 0.47	1.00 ± 0.00	1.00 ± 0.00
Losses:				
Preimplantation	0.13 ± 0.09	0.17 ± 0.18	0.01 ± 0.07	0.10 ± 0.08
Postimplantation	0.21 ± 0.39	0.11 ± 0.11	0.10 ± 0.11	0.05 ± 0.10
Fetuses/litter	10.57 ± 2.27	10.75 ± 2.36	10.94 ± 1.65	10.94 ± 2.34
		Adult		
Potency	0.60 ± 0.34	0.37 ± 0.30	0.66 ± 0.27	0.79 ± 0.24*
Fertility	0.89 ± 0.12	0.78 ± 0.35	0.88 ± 0.16	0.96 ± 0.05
Fecundity	0.90 ± 0.31	0.55 ± 0.52	1.00 ± 0.00*	1.00 ± 0.00*
Losses:				
Preimplantation	0.12 ± 0.12	0.08 ± 0.08	0.11 ± 0.16	0.03 ± 0.05
Postimplantation	0.06 ± 0.08	0.04 ± 0.09	0.03 ± 0.07	0.07 ± 0.05
Fetuses/litter	9.62 ± 3.81	7.77 ± 5.96	10.95 ± 2.71	10.66 ± 1.69

* Statistically different vs same age TT.
† Statistically different vs same age SH.

RESULTS

Age at TT Onset and Influence on Adult Testicle Function and Morphology

No statistical difference was found among the groups in the fertility test parameters investigated. Table 1 lists the numerical results of the fertility tests.

On spermatozoid analysis all rats with TT had lower concentration, motility and viability than SH rats regardless of age. TT9R samples completely lacked of spermatozoids. However, statistical analysis of the spermatozoid concentration indicated no difference among the TT groups. Since there were no spermatozoids in TT9R specimens, motility and viability analyses were performed only in TT4R and TT6R samples with no statistical difference noted. Table 2 lists the numerical results of spermatozoid analyzed parameters.

Testicular volume and weight also did not differ among TT4R, TT6R and TT9R specimens. However, the seminiferous epithelium histological parameters Av and Vv were greater in TT6R than in TT4R or TT9R samples (fig. 1). Table 3 shows the numerical results of morphological analysis.

Antioxidant Treatment Influence on TT

Fertility tests demonstrated some improvement in the groups treated with antioxidants. RES4 and RES6 rats showed 52% and 142% increased potency, respectively, compared to untreated rats. The ARG4, ARG6 and ARG9 groups also showed an increase in this parameter (66%, 187% and 113%, respectively). Interestingly, in ARG6 rats potency was even greater than in rats of the same age with SH. Each antioxidant also improved fecundity in TT9 rats.

Protection was considered total when TT results differed from SH results and antioxidant results were similar to those of SH but differed from TT results. Protection was considered partial when TT results differed from SH results and antioxidant results were similar to those in the TT and SH groups.

TT induced a marked decrease in spermatozoid production and quality in torsed testicles regardless of age at TT. Spermatic damage was not prevented by antioxidant treatment. RES6R spermatozoid viability was the only improvement associated with antioxidant treatment. In this group results revealed partial protection since viability did not statistically differ from that in SH6R or TT6R samples. Interestingly, each antioxidant improved contralateral spermatozoid quantity and quality.

TT induced a 46% to 64% decrease in testicular weight and volume regardless of age at TT. Resveratrol treatment prevented this decrease in the TT4 group while arginine provided partial protection from atrophy in TT6 and TT9 rats.

Resveratrol prevented changes in tubular lumen Av, blood vessel Vv and total tubular length in RES4R samples (fig. 2). In RES9R samples resveratrol prevented cellular proliferation in the interstitial space (fig. 3). It was also partially effective in preventing changes in tubular compartment Av, intertubular compartment Vv and tubular cellular

Table 2. *Spermatozoid analysis by age at onset*

	Mean ± SD Rt Testis			Mean ± SD Contralat Testis		
	Concentration (10^6/ml)	% Motility	% Viability	Concentration (10^6/ml)	% Motility	% Viability
Prepubertal:						
SH	4.6 ± 2.1	51.1 ± 16.2	18.7 ± 5.0	5.6 ± 4.0	44.4 ± 7.0	29.1 ± 7.8
TT	0.1 ± 2.1*	10.6 ± 18.0*	3.20 ± 6.74*	3.9 ± 2.5	53.9 ± 15.2	26.1 ± 6.24
RES	0.6 ± 1.2*	12.0 ± 18.5*	1.35 ± 2.94*	8.9 ± 6.3†	44.4 ± 7.01	21.1 ± 3.12
ARG	2.0 ± 3.6*	10.3 ± 11.9*	6.1 ± 8.6*	8.9 ± 3.9†	41.0 ± 8.1	40.0 ± 8.7†
Pubertal:						
SH	4.0 ± 2.1	49.5 ± 15.5	22.7 ± 11.3	4.5 ± 1.8	57.1 ± 16.0	24.6 ± 11.2
TT	1.1 ± 1.7*	18.0 ± 28.3*	7.16 ± 10.0*	5.0 ± 3.0	50.4 ± 23.6	21.6 ± 8.23
RES	0.4 ± 1.0*	12.3 ± 23.3*	8.7 ± 16.2	8.4 ± 2.9*	68.6 ± 8.3	33.9 ± 3.11
ARG	0.4 ± 0.7*	14.4 ± 25.3*	9.0 ± 10.7†	6.5 ± 3.8	71.4 ± 9.6	34.4 ± 4.1
Adult:						
SH	6.2 ± 5.6	41.7 ± 20.1	16.5 ± 4.34	8.6 ± 4.5	42.1 ± 10.5	17.5 ± 3.59
TT	0.00 ± 0.00*	Not assessed	Not assessed	6.9 ± 2.4	47.1 ± 10.5	18.5 ± 9.35
RES	0.8 ± 2.3*	7.1 ± 15.2*	5.35 ± 12.4*	7.5 ± 2.1	36.7 ± 8.5	31.9 ± 5.6‡
ARG	0.4 ± 0.6*	6.6 ± 9.5*	4.5 ± 4.8*	6.9 ± 2.5	48.0 ± 22.4	33.9 ± 7.86‡

* Statistically different vs same age SH.
† Statistically different vs same age TT.
‡ Statistically different vs same age SH and TT.

proliferation in RES4R samples. In RES6R specimens resveratrol partially prevented changes in intertubular compartment Vv. In RES9R samples resveratrol partially prevented the epithelial height reduction. However, resveratrol had a negative effect on some parameters. It decreased tunica propria Vv in testicles torsed at ages 4 and 6 weeks, and also decreased the tubular compartment Vv in testicles torsed at age 6 weeks.

Arginine partially prevented changes in seminiferous tubule diameter, seminiferous epithelium height, Vv and Av, blood vessel Vv, tunica propria Av and tubular compartment cellular proliferation in ARG4R specimens. In ARG9R samples arginine prevented increased cellular proliferation in the interstitial space. In ARG9R specimens it also partially prevented a decrease in epithelial height. However, arginine had a negative effect on some parameters. It decreased tunica propria Vv and blood vessel Av in ARG4R samples, decreased seminiferous epithelium Vv and Av in ARG6R samples, and increased blood vessel Vv in ARG9R samples. Table 4 shows the histological parameters protected by antioxidant treatment.

DISCUSSION

To our knowledge we evaluated for the first time all of certain variables together, including the TT effect on morphological, spermatic and fertility parameters. We compared rats of different ages and the effect of treatment with 2 different antioxidants.

Although TT may occur at any age, these age related prognoses have never been reported previously.[11] Our results revealed a significant decrease in sperm concentration without a statistical difference by age at TT onset. Nevertheless, adults were most affected by TT with azoospermia apparent in the torsed testis. Morphological data support these findings since TT9R specimens showed almost complete epithelial loss. The epithelium of pubertal rats was least affected by TT.

Also, spermatozoid parameters in TT6 rats were higher than in TT4 and TT9 rats, although this

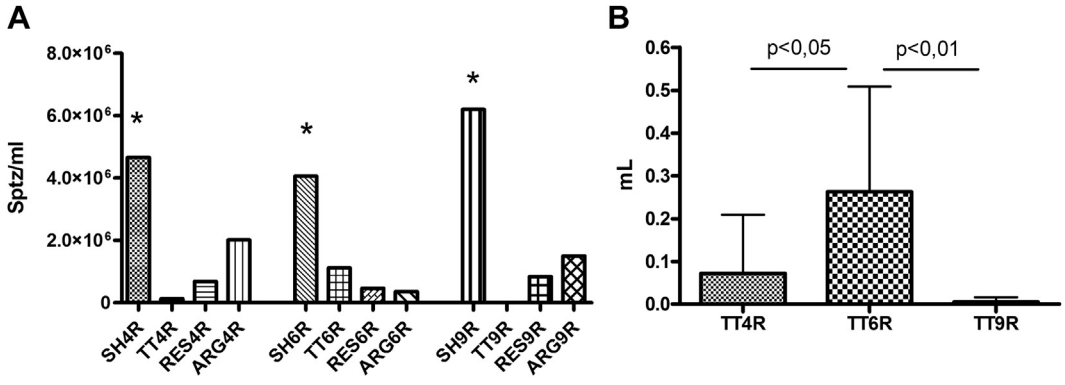

Figure 1. *A*, Mean ± SD spermatozoid (*Sptz*) concentration in SHR, TTR, RESR and ARGR epididymal tail. Asterisk indicates significant difference between SHR and other groups at same age. *B*, Mean ± SD Av of right testis seminiferous epithelium.

Table 3. *Right testis histological analysis*

	Mean ± SD Prepubertal				Mean ± SD Pubertal				Mean ± SD Adult			
	SH	TT	RES	ARG	SH	TT	RES	ARG	SH	TT	RES	ARG
Seminiferous tubular diameter (μm)	270 ± 17.9	166 ± 61.0*	182 ± 59.6*	230.3 ± 85.63*	300 ± 21.8	198 ± 89.2*	202 ± 15.0*	204.7 ± 41.95*	312 ± 12.1	217 ± 23.3*	232 ± 23.0*	203.7 ± 15.47*
Seminiferous epithelium:												
Ht (μm)	47.6 ± 5.81	15.3 ± 20.7*	27.9 ± 12.4*	26.59 ± 20.45	48.3 ± 7.56	15.6 ± 19.8*	23.5 ± 15.3*	17.14 ± 13.86*	54.7 ± 2.63	0.00 ± 0.00*	17.4 ± 20.5	20.8 ± 15.5*
% Vv	32.3 ± 5.30	8.75 ± 11.9*	8.97 ± 9.84*	15.23 ± 18.07	45.3 ± 4.95	25.9 ± 17.5*	8.56 ± 13.5*	6.23 ± 8.84*,†	39.4 ± 5.86	0.84 ± 1.78*,§	3.68 ± 10.5*	0.04 ± 0.14*
Av (ml)	0.45 ± 0.06	0.07 ± 0.13*	0.09 ± 0.10*	0.275 ± 0.324	0.69 ± 0.06	0.26 ± 0.24*,‡	0.08 ± 0.11*	0.06 ± 0.10*,†	0.55 ± 0.26	0.00 ± 0.01*,§	0.01 ± 0.04*	0.005 ± 0.011*
Tunica propria:												
% Vv	6.67 ± 2.06	5.83 ± 1.83	3.12 ± 0.75*,†	3.31 ± 1.74*,†	6.66 ± 1.95	5.76 ± 0.93	3.42 ± 0.58*,†	4.87 ± 1.26	5.19 ± 1.08	3.72 ± 2.53	3.30 ± 1.53	3.30 ± 0.52
Av (ml)	0.09 ± 0.03	0.03 ± 0.03*	0.03 ± 0.01*	0.06 ± 0.030	0.10 ± 0.02	0.04 ± 0.01*	0.02 ± 0.01*	0.03 ± 0.02*	0.07 ± 0.03	0.02 ± 0.02*	0.02 ± 0.01*	0.03 ± 0.017*
Tubular lumen:												
% Vv	32.6 ± 7.13	33.2 ± 6.37	35.8 ± 13.3	29.60 ± 14.19	38.6 ± 7.76	38.8 ± 11.4	45.9 ± 8.07	46.18 ± 13.60	40.4 ± 7.32	42.7 ± 28.5	37.2 ± 15.2	40.07 ± 15.66
Av (ml)	0.47 ± 0.13	0.15 ± 0.14*	0.46 ± 0.21§	0.54 ± 0.25§	0.60 ± 0.16	0.32 ± 0.18*	0.37 ± 0.11*	0.33 ± 0.20*	0.53 ± 0.27	0.31 ± 0.23	0.30 ± 0.17	0.36 ± 0.24
Tubular compartment:												
% Vv	71.6 ± 7.90	47.8 ± 17.0*	52.9 ± 9.71*	48.15 ± 28.08*	90.6 ± 2.98	73.4 ± 17.9*	57.9 ± 12.3*	57.28 ± 18.52*	85.6 ± 1.76	47.3 ± 31.2*	44.2 ± 20.8*	48.40 ± 5.051*
Av (ml)	1.02 ± 0.18	0.25 ± 0.31*	0.58 ± 0.29	0.88 ± 0.49§	1.39 ± 0.14	0.63 ± 0.43*	0.47 ± 0.17*	0.43 ± 0.30*	1.17 ± 0.55	0.34 ± 0.26*	0.34 ± 0.26*	0.39 ± 0.26*
Intertubular compartment:												
% Vv	8.23 ± 2.85	36.5 ± 20.5*	24.2 ± 13.4	35.71 ± 26.65*	9.34 ± 2.98	28.8 ± 17.2*	19.1 ± 13.8	25.80 ± 18.36*	14.9 ± 1.93	52.6 ± 31.2*	39.8 ± 22.7*	40.63 ± 21.07*
Av (ml)	0.11 ± 0.03	0.15 ± 0.25	0.29 ± 0.11	0.71 ± 0.51*,†	0.14 ± 0.04	0.17 ± 0.05	0.18 ± 0.21	0.16 ± 0.13	0.20 ± 0.10	0.40 ± 0.44	0.35 ± 0.19	0.37 ± 0.20
Blood vessel:												
% Vv	0.87 ± 0.57	4.37 ± 3.99*	1.51 ± 0.94§	2.533 ± 1.187	1.68 ± 1.02	3.36 ± 1.91	1.90 ± 1.26	2.00 ± 0.91	1.10 ± 0.34	2.26 ± 0.75	3.24 ± 1.53*	2.13 ± 0.61*
Av (ml)	0.01 ± 0.00	0.01 ± 0.03	0.01 ± 0.00	0.046 ± 0.0211*,†	0.02 ± 0.01	0.02 ± 0.00	0.01 ± 0.01	0.01 ± 0.009	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.018 ± 0.009
Tubular length (m)	18.0 ± 4.45	9.29 ± 7.11	18.3 ± 4.01	13.01 ± 13.36	19.6 ± 3.01	9.63 ± 7.28*	7.41 ± 2.41*	12.63 ± 7.63	16.1 ± 7.63	10.4 ± 7.15	9.80 ± 5.77	13.68 ± 7.91
Compartment cellular proliferation (cells/mm^2):												
Tubular	1.3×10^3 ± 439	343 ± 426*	716 ± 476	841 ± 658	1.2×10^3 ± 230	723 ± 394	403 ± 498	293.2 ± 489.0*	1.7×10^3 ± 424	267 ± 233*	308 ± 570*	168.8 ± 226.8*
Intertubular	349 ± 454	601 ± 291	714 ± 718	236.4 ± 162.9	874 ± 686	821 ± 247	398 ± 273	175.9 ± 123.2*,†	401 ± 191	828 ± 219*	260 ± 364†	173.8 ± 109.7†

* Statistically different vs same age SH.
† Statistically different vs same age TT.
‡ Statistically different vs prepubertal TT.
§ Statistically different vs pubertal TT.

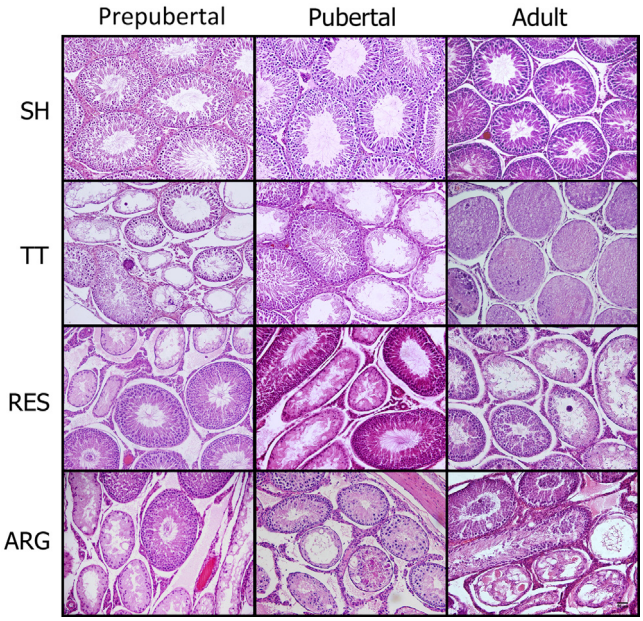

Figure 2. Photomicrographs show right testis seminiferous tubular damage in SH, TT, RES and ARG prepubertal, pubertal and adult rats. H&E, reduced from ×200.

difference was not statistically significant. This indicates that when TT occurs during puberty, the prognosis is better than in earlier or later life.

These results suggest that more effort should be given to decrease the time from the beginning of ischemia to surgical resolution in adults who present with TT. Also, more research about adjuvant therapies that could ameliorate testicular damage would be of special importance for adults.

Spermatozoid parameters were not statistically improved by antioxidant treatment. However, in rats that underwent TT in adulthood each drug aided in the recovery of some viable spermatozoids in the torsed testis, which seems promising for clinical use.

Rats submitted to TT before puberty were most benefited by antioxidants, showing significant protection of tubular structures. In contrast, treatments were of little help in rats operated on during puberty. It is difficult to explain these findings but the pubertal testicle, which is in a changing state, may possibly be more tolerant to disruption while prepubertal testicles would be more susceptible to oxidative stress and, thus, be more protected by antioxidants. Regardless of the mechanisms of how these antioxidants act on TT, they could help preserve testicular function. This may have a more prominent effect in infants.

Although TT did not affect fertility in any rat, antioxidant treatment promoted improved fecundity and potency. Antioxidants also improved some spermatozoid parameters of the contralateral testis.

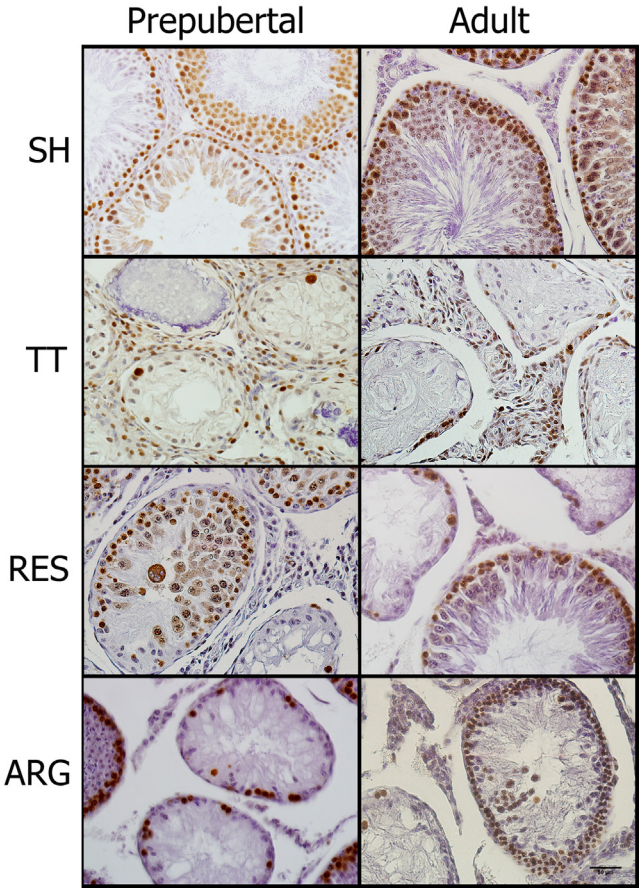

Figure 3. Photomicrographs reveal proliferating cells in tubular and interstitial spaces of right testis seminiferous tubules in SH, TT, RES and ARG prepubertal and adult rats. Proliferation cell nuclear antigen immunostaining, reduced from ×400.

Nitric oxide protects the contralateral testis after TT,[22,23] which could explain the positive effects of arginine and resveratrol on the contralateral testis since each enhances nitric oxide bioavailability.[13,24]

Table 4. *Histological parameters partially or totally protected by antioxidants*

	Protection*				
	RES4	RES6	RES9	ARG4	ARG9
Seminiferous tubular diameter	—	—	—	Partial	—
Seminiferous epithelium:					
Ht	—	—	Partial	Partial	Partial
Vv	—	—	—	Partial	—
Av	—	—	—	Partial	—
Tunica propria Av	Total	—	—	Partial	—
Tubular:					
Length	Total	—	—	Partial	—
Lumen Av	Total	—	—	Partial	—
Compartment Av	Partial	—	—	Partial	—
Intertubular compartment Vv	Partial	Partial	—	—	—
Blood vessel Vv	Total	—	Partial	Partial	—
Compartment cellular proliferation:					
Tubular	Partial	—	—	Partial	—
Intertubular	—	—	Total	—	Total

* ARG6 rats were not protected.

A limitation of this study is the anatomy of the rat spermatic cord, which is thinner, longer and fatter than the human spermatic cord. This could minimize the effects of TT but ischemia was visually confirmed in all rats with TT. The suture applied to the testicle could promote testicular injury.[25,26] However, we used a SH group to avoid this bias. We also evaluated all rats at age 14 weeks. Thus, the time between TT and the assessment of its effects differed in each group. This was done intentionally to assess the impact of TT in adulthood when TT occurred at different ages.

CONCLUSIONS

Age at TT did not influence spermatozoid production or fertility in adulthood. However, testicular morphology was less affected in rats that underwent TT during puberty. Treatment with resveratrol or arginine did not enhance spermatozoid parameters in the torsed testicle but it improved the contralateral testicle and some fertility parameters. Each antioxidant also ameliorated testicular morphology after TT. Prepubertal rats benefited most after each antioxidant treatment.

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EDITORIAL COMMENT

The authors add to the growing body of literature on the beneficial effects of antioxidants in the setting of testicular torsion/detorsion. Restoring blood flow is associated with the generation of free radicals. Antioxidants truncate the damaging chain reactions that follow. The current study is the first to investigate antioxidants for prepubertal, pubertal and postpubertal testicular torsion in the rat.

Torsion/detorsion reduced the sperm concentration in all groups but the adult group was the only azoospermic cohort. It is possible that this finding represents the increased vulnerability of the adult testis but also possible that the finding stems from the fact that this group had a shorter interval between torsion and evaluation. Similarly, the noted improvement in tubular structures in the prepubertal group could potentially be related to a longer recovery period. Potency was improved by antioxidant therapy, a finding consistent with the fact that each agent increases nitric oxide, as the authors note. No statistically significant fertility benefit was observed in any group. Fecundity was improved in treated adults. The contralateral testis and not the twisted testis may be the primary beneficiary of antioxidants. Each antioxidant statistically improved the quantity and quality of spermatozoid from the contralateral but not the ipsilateral testis.

Other potential interventions include cyclooxygenase-2 inhibition,[1] sildenafil[2] and short-term post-conditioning (clamping spermatic vessels for 5-second intervals).[3] Finally, hypothermia has proved to decrease reperfusion associated free radical formation in patients with brain injury.[4] Perhaps we should apply ice before and after testicular detorsion.

Dan McMahon
Akron Children's Hospital
Northeast Ohio Medical University
Rootstown, Ohio

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