Long-term effects of the testicular torsion on the spermatogenesis of the contralateral testis and the preventive value of the twisted testis orchiepididymectomy[1]

Efeitos tardios da torção testicular sobre a espermatogênese do testículo contralateral e o valor preventivo da orquiepididimectomia do testículo torcido

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ABSTRACT

PURPOSE: To determine whether the testicular torsion causes long-term effects on the spermatogenesis of the contralateral testis, and whether the orchiepididymectomy of the twisted testis could prevent them, using specific spermatogenesis parameters to elucidate the conflicting results in the literature.

METHODS: Seventy-four pubertal male Wistar rats were randomly selected. The experimental group consisted of 40 rats, divided into four subgroups, submitted to 1.080 degrees counterclockwise left testicular torsion and its scrotal fixation at the beginning of the experiment, and left orchiepididymectomy at one, five, ten and 90 days, respectively. The control group consisted of 24 rats, divided into four sham operation control subgroups. An additional control subgroup consisted of the ten remaining rats, submitted only to the left orchiepididymectomy at the beginning. At 90 days, the contralateral testes of the experimental and control subgroups were collected for the evaluation of their spermatogenesis parameters: testicular weight, seminiferous tubular diameter, Johnsen score and differential counting of the germ cells.

RESULTS: No statistically significant differences were observed among the experimental and control subgroups for all of the spermatogenesis parameters of the contralateral testes.

CONCLUSIONS: Testicular torsion does not cause long-term effects on the spermatogenesis of the contralateral testis in pubertal rats, and the orchiepididymectomy of the twisted testis is not necessary for preventive purposes for the contralateral spermatogenesis.

Key words: Spermatic Cord Torsion. Spermatogenesis. Testis. Rats.

RESUMO

OBJETIVO: Determinar se a torção testicular causa efeitos tardios sobre a espermatogênese do testículo contralateral e se a orquiepididimectomia do testículo torcido poderia preveni-los, usando parâmetros específicos da espermatogênese para elucidar os resultados conflitantes na literatura.

MÉTODOS: Foram selecionados aleatoriamente 74 ratos machos púberes da linhagem Wistar. O grupo experimental foi composto por 40 ratos divididos em quatro subgrupos, submetidos à torção anti-horária de 1,080 graus do testículo esquerdo e sua fixação escrotal no início do experimento e à orquiepidimectomia esquerda com um, cinco, dez e 90 dias, respectivamente. O grupo controle foi composto por 24 ratos divididos em quatro subgrupos de cirurgias simuladas. Um subgrupo controle adicional foi constituído pelos dez ratos restantes submetidos unicamente à orquiepididimectomia esquerda no início do experimento. Aos 90 dias, os testículos contralaterais dos subgrupos experimentais e controles foram coletados para avaliação dos parâmetros de suas espermatogêneses: peso testicular, diâmetro do túbulo seminífero, graduação de Johnsen e contagem diferencial das células germinativas.

RESULTADOS: Não houve diferença estatisticamente significativa entre todos os subgrupos experimentais e controles para todos os parâmetros analisados da espermatogênese dos testículos contralaterais.

CONCLUSÕES: A torção testicular não causa efeitos tardios sobre a espermatogênese do testículo contralateral em ratos púberes e a orquiepididimectomia do testículo torcido não é necessária para fins preventivos da espermatogênese contralateral.

Descritores: Torção do Cordão Espermático. Espermatogênese. Testículo. Ratos.

Introduction

Testicular torsion is the rotation of the testis with its epididymis and spermatic cord around its own longitudinal axis. It is the most important scrotal emergency because the high risk of permanent lesions to the twisted and to the contralateral testis[1-3]. It is a common urological emergency during puberty, with annual incidence of one in 4.000 males under 25 years of age[4], occurring exactly at the same time as when the boy is processing his psychological sex self-affirmation and the spermatogonium starts its development to form spermatozoids. Its aetiology is usually due to an anatomical defect, frequently bilateral, caused by the upward extension of the tunica vaginalis insertion on the spermatic cord, so that the testis is suspended freely inside the tunical cavity, allowing it to rotate. For this reason, the peak of incidence of testicular torsion is around puberty, just when the testicular mass is rapidly increasing, predisposing it to torsion[5].

The immediate diagnosis and treatment aims to minimize the testicular lesions[1]. However, the testicular torsion is frequently misdiagnosed as orchiepididymitis, or the patient takes too long to seek medical attention, resulting in irreversible spermatogenesis damage, testicular atrophy and low fertility in adulthood[1,3].

Because the torsion of the testicle usually causes severe and permanent damage to the ipsilateral spermatogenesis due to the necrosis of their germ cells, the preservation of the spermatogenesis of the contralateral testis is essential to preserve the fertility of the patient.

It is well-established in the literature that testicular torsion causes early damage to the spermatogenesis of the contralateral testis[6-9]. However, there are few and conflicting results in the literature as to whether the testicular torsion causes permanent damage (long-term effects) to the spermatogenesis of the contralateral testis, and whether the orchiepididymectomy of the twisted testis could prevent this damage[10-15]. Many theories have been suggested to explain the contralateral injuries to the spermatogenesis, such as auto-immunization induced by the necrotic haploid germ cells of the twisted testis[16], production of reactive oxygen species[11,14] and contralateral sympathetic reflex[13].

Information regarding long-term effects on the contralateral spermatogenesis, and the value of the orchiepididymectomy of the twisted testis, would be very useful to guide two important and common circumstances in the surgical management of testicular torsion: first, when the twisted testis shows a partial or insufficient reperfusion during its surgical repair, the question is whether this testis should be left inside the scrotum, or removed to prevent or minimize the late spermatogenesis damage to the contralateral testis; second, in cases in which the elapsed time from the beginning of the testicular torsion until medical evaluation is so long that the patient became asymptomatic and the testicle is presumably unviable, the question is whether its orchiepididymectomy should still be performed for preventive purposes for the contralateral spermatogenesis.

The objectives of this study are to determine whether the testicular torsion causes long-term effects to the spermatogenesis of the contralateral testis, and whether the orchiepididymectomy of the twisted testis could prevent them, by using specific spermatogenesis parameters in order to elucidate the conflicting results in the literature.

Methods

This study was previously approved by the Research Ethics Committee of Evangelical Society of Curitiba, Brazil, under protocol No. 8734/08.

Seventy-four pubertal male Wistar rats, aged 45 to 50 days and with a minimum body weight of 130g, were used.

The animals were anesthetized intraperitoneally with ketamine (50mg/Kg) and xylazine (10mg/kg) for all surgical procedures, followed by postoperative analgesia with meloxicam and sodium dipyrone[17]. For all surgical procedures, under sterile conditions, a left or right longitudinal scrotal incision was performed to access the left or right testis and epididymis, respectively, which was closed with 4/0 catgut plain sutures. All procedures were performed in the morning periods to avoid any influence of circadian rhythm of testosterone.

Experimental and control groups

The experimental group consisted of 40 rats randomly divided into four experimental subgroups (ESG), each with ten rats (ESG.01, ESG.05, ESG.10 and ESG.90) submitted to left testicular torsion of 1.080 degrees in a counterclockwise direction and its left testicular fixation inside the scrotum with 5/0 polyamide monofilament sutures at the beginning of the experiment (Figure 1), and the left testis and epididymis resection (left orchiepididymectomy) at one, five, ten and 90 days, respectively (Chart 1).

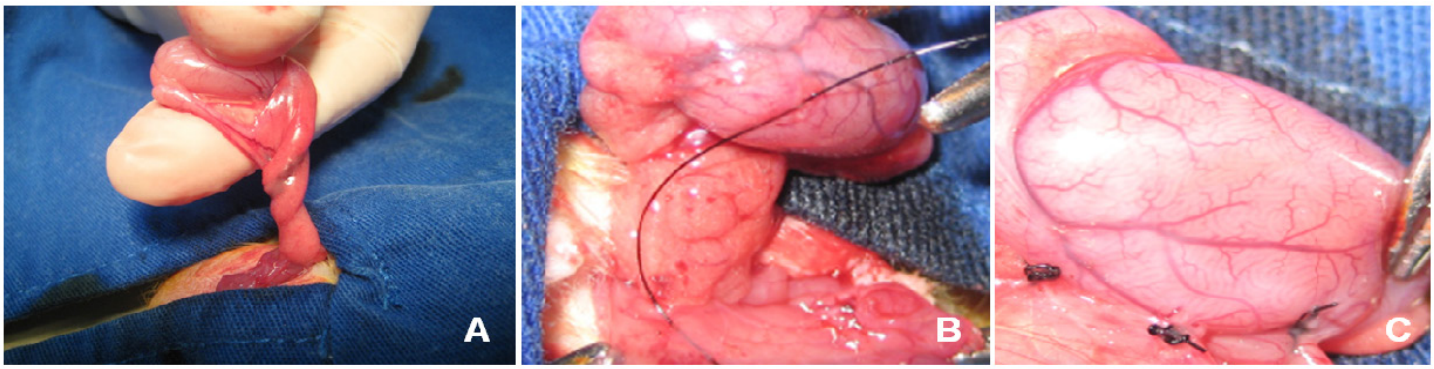

FIGURE 1 - Photographs of the experimental group: **A**) Left testicular torsion of 1.080 degrees in a counterclockwise direction, **B**) 5/0 polyamide monofilament suture, placed through the testicular albuginea and medial scrotal wall, to fix the left twisted testis, **C**) Three fixation stitches of the torsion (black color).

CHART 1 - Experimental subgroups design of the study.

Subgroups	Initial procedure	01 day	05 days	10 days	90 days
ESG.01 (n=10)	Left testicular torsion and its scrotal fixation	Left orchi-epididymectomy	-	-	Right orchiectomy
ESG.05 (n=10)		-	Left orchi-epididymectomy	-	
ESG.10 (n=10)		-	-	Left orchi-epididymectomy	
ESG.90 (n=10)		-	-	-	Right orchiectomy and left orchi-epididymectomy

ESG.n = Experimental subgroups: the rats were submitted to the left testicular torsion and its fixation inside the scrotum at the beginning of the experiment, and the left orchiepididymectomy at n days. At 90 days, the rats were submitted to the right orchiectomy.

The control group included four control subgroups of sham operations (CSG.SO), each with six rats (CSG.SO.01, CSG. SO.05, CSG.SO.10 and CSG.SO.90), submitted only to the left testicular fixation inside the scrotum (left orchiopexy) at the initial of the experiment and the left testis and epididymis resection (left orchiepididymectomy) at one, five, ten and 90 days, respectively (Chart 2).

An additional control subgroup (CSG.LO) consisted of the remaining ten rats, submitted only to the left testis and epididymis resection (left orchiepididymectomy) at the beginning of the experiment (Chart 2).

CHART 2 - Control subgroups design of the study.

Subgroups	Initial procedure	01 day	05 days	10 days	90 days
CSG.SO.01 (n=06)	Left orchiopexy	Left orchi-epididymectomy	-	-	Right orchiectomy
CSG.SO.05 (n=06)		-	Left orchi-epididymectomy	-	
CSG.SO.10 (n=06)		-	-	Left orchi-epididymectomy	
CSG.SO.90 (n=06)		-	-	-	Left orchi-epididymectomy and right orchiectomy
CSG.LO (n=10)	Left orchi-epididymectomy	-	-	-	Right orchiectomy

CSG.SO.n = Control subgroups of sham operations: the rats were submitted to the left testicular fixation inside the scrotum (left orchiopexy) at the beginning of the experiment and left orchiepididymectomy at n days; CSG.LO = Control subgroup of left orchiepididymectomies: the rats were submitted only to the left orquiepididymectomy at the initial of the experiment; at 90 days, the rats were submitted to the right orchiectomy.

At 90 days, all of the experimental and control subgroups rats were weighed and underwent right orchiectomies (Charts 1 and 2).

Histological processing

The left and right testes, at the time of their resections, were submitted to the resection of four fragments of approximately 2x2x2 mm from their testicular parenchyma, which were immediately immersed for four hours in Bouin's solution, washed in water and fixed for 24 hours in 10% formalin solution. Two of these fragments were embedded in paraffin, cut into four micron-thin cross-sections, and stained with hematoxylin and eosin (HE), while the other two fragments were stored as back-up for the research.

Evaluation of the contralateral testes parameters

The right contralateral testes were evaluated for their spermatogenesis parameters: testicular weights, seminiferous tubular diameters, Johnsen scores[18] and differential counting of the germ cells: round spermatids and primary spermatocytes in pachytene per seminiferous tubule. The final value of each right testis histological parameter was the average of their measurements in ten cross-sections, evaluated at stages VII and VIII of the seminiferous epithelium cycle (SEC).

Determination of the seminiferous tubular diameters and the cell counts were performed using the histological images digitalized by the ImageTool 3.0 Software for Windows. All of the analysis was carried out by a single pathologist, blinded to experimental manipulations. The cell count numbers were

corrected by Abercrombie's formula[19,20] for the thickness of the histological section and the diameter of the germ cell nucleus.

Statistical analysis

The parameters of the right contralateral testes were statistically analyzed: a) among the subgroups ESG.01, ESG.05, ESG.10, ESG.90, CSG.SO (ten combinations): to determine whether the testicular torsion caused long-term effects on the spermatogenesis of the contralateral testis and to determine whether the orchiepididymectomy of the twisted testis could prevent them, b) among the subgroup CSG.LO and the subgroups ESG.01, ESG.05, ESG.10, ESG.90, CSG.SO (five combinations): to evaluate whether the single left orchiepididymectomy at the beginning of the experiment caused the same long-term effects on the spermatogenesis of the contralateral testis as the left orchiepididymectomy of the twisted testis in the experimental subgroups and/or as the sham operations.

According to the sampling characteristics of this research, all of the parameters of the spermatogenesis of the contralateral testes were analyzed by the Mann-Whitney non-parametric independent sample test, adopting the statistical difference level of less than 5% ($p<0.05$).

Results

The rat weights at the beginning and at the end of the experiment, and their variations, were not statistically different among the research subgroups.

The macroscopic aspects of left twisted testes of the experimental subgroups at one, five, ten and 90 days are shown in Figure 2, showing the different colors of the surface of the testis, according to the duration of its torsion, and its hypotrophy at 90 days.

The histological aspects of the left testes collected one day after its torsion showed morphologically preserved cells in the periphery of the seminiferous tubules and necrotic anucleated and exfoliated cells in their lumens. At five days of torsion, the cells were completely necrotic, exfoliated and anucleated, with the normal shape of the tubules still maintained. At ten days, the seminiferous tubules were totally necrotic with onset of phagocytosis by the leukocytes in the periphery of the tubules. At 90 days, the testes were completely replaced by collagen, with the presence of capillaries and fibroblasts (Figure 3).

At 90 days, all right contralateral testes showed normal macroscopic aspects, and their weights and histological parameters are summarized in Table 1. The control subgroups of the sham operations, CSG.SO.01, CSG.SO.05, CSG.SO.10 and CSG.SO.90, were grouped as only one subgroup of 24 rats (CSG.SO) because their statistical analysis showed no significant differences among them for all of the parameters (Mann-Whitney test, $-1.96 \leq z \leq 1.96$, $p>0.05$).

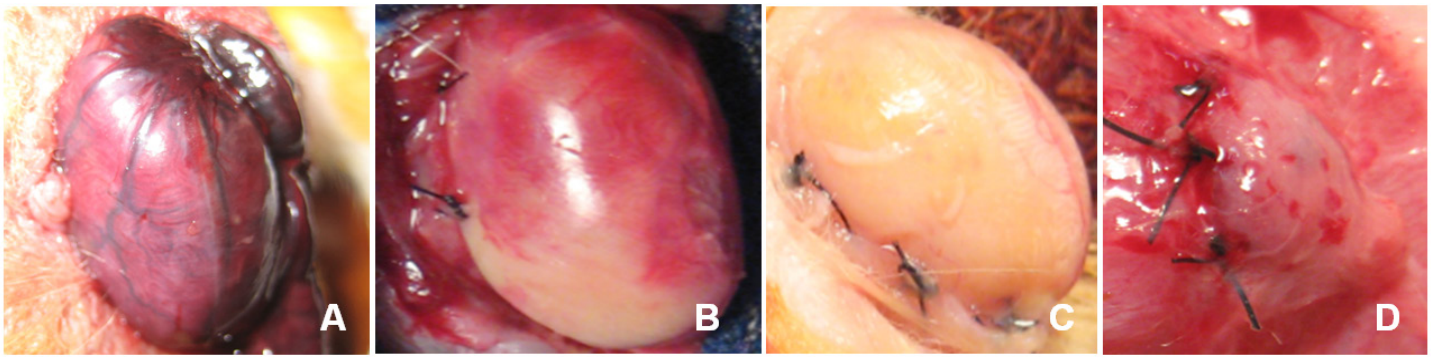

FIGURE 2 - Photographs A, B, C and D show the macroscopic aspects of the twisted left testes of the experimental subgroups at one, five, ten and 90 days, respectively: **A**) Dark red color due to the ischemia, **B**) Partially white-yellow color, **C**) Completely white-yellow color, **D**) Hypotrophy and normal color.

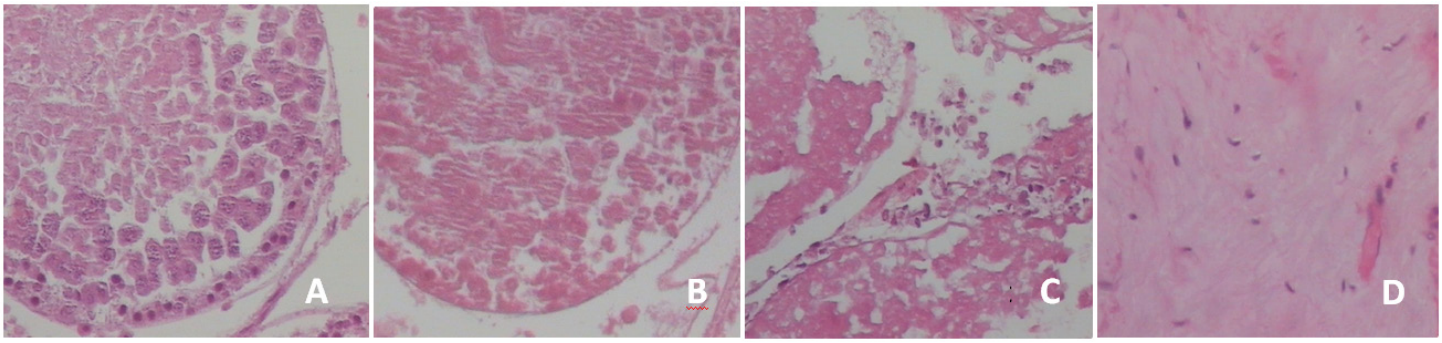

FIGURE 3 - Figures A, B, C and D show the histological findings of the twisted left testes collected at one, five, ten and 90 days, respectively (HE, 200X): **A**) Preserved cells in the periphery of the seminiferous tubules and their lumens with necrotic anucleated and exfoliated cells; **B**) Completely necrotic, exfoliated and anucleated cells, tubules still with the normal shape; **C**) Totally necrotic seminiferous tubules and activity of the leukocytes (phagocytosis); **D**) Testes completely replaced by collagen, with the presence of capillaries and fibroblasts.

TABLE 1 - Results of the parameters of the right contralateral testes spermatogenesis of the experimental and control subgroups.

Subgroups	n	RTW (g)[a] ($\bar{x} \pm s$)	STD (μ)[b] ($\bar{x} \pm s$)	Johnsen score[c] ($\bar{x} \pm s$)	NRS/ST[d] ($\bar{x} \pm s$)	NPSP/ST[e] ($\bar{x} \pm s$)
ESG.01	10	1.83 ± 0.12	283.05± 7.90	9.94 ± 0.10	70.59 ± 1.60	22.21 ± 0.90
ESG.05	10	1.80 ± 0.16	280.48 ± 5.14	9.91 ± 0.10	69.30 ± 2.28	22.53 ± 0.55
ESG.10	10	1.78 ± 0.10	277.88 ± 8.26	9.89 ± 0.14	70.83 ± 1.64	22.07 ± 0.84
ESG.90	10	1.79 ± 0.07	279.59 ± 4.53	9.93 ± 0.09	71.10 ± 1.94	22.39 ± 0.58
CSG.SO	24	1.84 ± 0.04	280.06 ± 3.36	9.93 ± 0.10	70.68 ± 1.45	22.45 ± 0.62
CSG.LO	10	1.82 ± 0.11	282.15 ± 5.19	9.92 ± 0.11	69.94 ± 1.88	22.43 ± 0.58

RTW = right testis weight; STD = seminiferous tubule diameter; NRS/ST = number of round spermatids per cross-section of the seminiferous tubule; NPSP/ST = number of primary spermatocytes in pachytene per cross-section of the seminiferous tubule; $\bar{x} \pm s$ = mean ± standard deviation.
[a,b,c,d,e] No statistically significant differences were observed among the experimental and control subgroups for all of the parameters (Mann-Whitney test, $-1.96 \leq z \leq 1.96$, $p>0.05$).

The statistical analysis of all of the parameters of the spermatogenesis of the contralateral testes showed no significant differences among the experimental and control subgroups (Mann-Whitney test, $-1.96 \leq z \leq 1.96$, $p>0.05$).

Discussion

In order to be similar to the most frequent characteristics of human testicular torsion: pubertal age, internal torsion and left side[21,22], this research used pubertal rats and counterclockwise torsion direction of the left testis.

The intensity of testicular damage is directly proportional to the degree and duration of the testicular torsion[16,23]. So, this study used a high level of 1.080 degrees of left testicular torsion, for different periods of time, to exacerbate the damage to the spermatogenesis of both testes. This high degree of torsion exhibited the expected histological stages of the coagulation necrosis of the ipsilateral testicular parenchyma (Figure 3), as we already observed in a previous study[24] using the same degree of testicular torsion in adult rats.

The length of one cycle of the seminiferous epithelium cycle (SEC) lasts 12.9 days in the rat and approximately 58 days (4.5 cycles) are required for the committed type A spermatogonia to differentiate into mature spermatozoa[19,25]. Therefore, the period of 90 days used in the present study can be considered adequate to detect the long-term injuries induced on the undifferentiated spermatogonia, as well as the possible recovery of the seminiferous epithelium of the contralateral testis.

There are few experimental studies in the literature in regard to the long-term effects of testicular torsion on the spermatogenesis of the contralateral testis, and the value of the orchiepididymectomy of the twisted testis to prevent them[10-15].

Using unilateral testicular torsion of 720 degrees in adult rats for one, two or four hours, Turner[10] observed no injury to the contralateral spermatogenesis testis after seven, 30 and 60 days, evaluating its testis weight, spermatogenesis status only

subjectively classified as normal or abnormal and the concentration and motility of the spermatozoids in the tail of the epididymis.

Prillaman and Turner[11] using the unilateral testicular torsion of 720 degrees in adult rats for one or two hours, found no effects on the weight and the estimated daily sperm production of contralateral testis at 60 days. The venous serum testosterone concentration also remained unchanged.

On the other hand, Kosar *et al.*[12] submitted adult rats to various degrees of unilateral testicular torsion for 12, 48 hours or three months, with or without orchiectomy of the twisted testis one month after its torsion. The contralateral testis at one month showed a significant decrease of the testicular weight, seminiferous tubular diameter and Johnsen score, with significant improvement at three months only in the group that underwent orchiectomy of the twisted testis, suggesting that the orchiectomy could be beneficial to prevent those alterations.

Tander *et al.*[13] performed a unilateral testicular torsion of 720 degrees for 55 days in prepubertal rats, verifying that the contralateral testis showed a statistically significant decrease of Johnsen scores, indicating spermatogenesis damage, although the seminiferous tubule diameter decreased with no statistical significance. Neither the ligation of the genito-femoral nerve nor the orchiectomy of the twisted testes on the fourth day prevented these injuries.

All of the above studies did not take into account the stages of the seminiferous epithelium cycle (SEC). However, the SEC is extremely important for the evaluation of spermatogenesis of the contralateral testis because each histological section of the seminiferous tubule displays different stages of the SEC and, furthermore, the majority of the 14 stages in the rat have no spermatozoids inside its lumen under physiological conditions[19]. In the present study, the stages VII and VIII were chosen because they have the most mature germ cells of the SEC in their seminiferous tubular lumens[19], i.e., spermatozoids, so these stages are the most sensitive in demonstrating any injuries to their spermatogenesis. Furthermore, the seminiferous tubular diameter varies depending on the thickness of each stage of the SEC, in this study its measurement was performed in these fixed VII and VIII stages to avoid any variation due to the stages of the SEC. The evaluation of SEC has also been used in the design of testing protocols to study the toxicological effects of drugs on the male reproductive system[25,26].

Vigueras *et al.*[14] performed the first study and probably the only one in the literature to use the concept of the stages of the SEC in testicular torsions, however this study used only qualitative parameters, i.e., the unilateral testicular torsion of 720 degrees for different periods of time in 60-days-old rats, analyzing the contralateral testis spermatogenesis parameters: degeneration, hypoplasia and loss of germ cells, degeneration of tubular basement membrane and presence of intraepithelial vacuoles, all of them without quantitative evaluations. After 60 days of the testicular torsion, the contralateral testis exhibited significant changes in all parameters, indicating the contralateral testicular damage of the spermatogenesis.

Sun *et al.*[15] observed that unilateral testicular torsion in prepubertal rats for two or six hours, or maintained until the postpubertal life, caused a significant decrease in the percentage of haploid cells of the contralateral testis in the postpubertal life, indirectly evaluated by the tissue DNA concentration. However, this concentration of DNA estimates only the sum of all germ cells, without quantifying the number of these different cells.

The literature remains in conflict as to whether the unilateral testicular torsion causes long-term effects on the spermatogenesis of the contralateral testis, and whether the orchiepididymectomy of the twisted testis could prevent them.

The present study using specific spermatogenesis parameters, including the counting of specific germ cells, taking into account the stages of the SEC, demonstrated that testicular torsion did not cause long-term effects on the spermatogenesis of the contralateral testis, and the orchiepididymectomy of the twisted testis is not necessary for preventive purposes for the contralateral spermatogenesis.

Based upon this research, further experimental investigations should be performed, such as the evaluation of whether testicular torsion, after a very long period of time (years), could induce a compensatory spermatogenesis function of the contralateral testis, and studies using infrared thermometry to evaluate the dynamic blood flow of the contralateral testis.

Conclusions

Testicular torsion does not cause long-term effects on the spermatogenesis of the contralateral testis in pubertal rats, and the orchiepididymectomy of the twisted testis is not necessary for preventive purposes for the contralateral spermatogenesis.

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