

## Propofol effects on the morphology of rat testes subjected to testicular ischemia-reperfusion<sup>1</sup>

Efeitos do propofol sobre a morfologia do testículo do rato submetido à isquemia-reperusão testicular

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### ABSTRACT

**PURPOSE:** To evaluate the effects of propofol as an inhibitor of tissue injury in testicular ischemia-reperfusion in rats.

**METHODS:** 30 Wistar rats were assigned to one of three groups of 10 animals: G1, testicular exposure alone; G2 and G3: testicular ischemia caused by left spermatic cord torsion of 720°. In G3, propofol was administered intraperitoneally at 20 mg/kg/h 45 minutes after spermatic cord torsion. In G2 and G3, spermatic cords were detorsioned after 60 min. In all three groups, testes were subsequently repositioned in the scrotum. After 90 days, bilateral orchiectomy was performed for histological examination.

**RESULTS:** No abnormalities in seminiferous tubules were found in G1. In G2, 86.6% of left testes exhibited abnormalities, in contrast with 67.8% for right testes. In G3, these proportions were 57.3% and 45.6%, respectively. A statistically significant difference was found between G2 and G3. **CONCLUSION:** Propofol reduced the tissue damage in rat testes subjected to ischemia-reperfusion caused by spermatic cord torsion.

**Key words:** Propofol. Testis. Ischemia. Reperfusion. Rats.

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### RESUMO

**OBJETIVO:** Avaliar os efeitos do propofol como inibidor da lesão tecidual na isquemia-reperusão testicular em ratos.

**MÉTODOS:** Trinta ratos Wistar foram distribuídos em três grupos de 10 animais. G1: apenas exposição testicular. G2 e G3: isquemia testicular por torção do cordão espermático esquerdo a 720°. G3, 45 minutos após a torção do cordão espermático foi administrado propofol 20mg/Kg/hora via intraperitoneal. Após 60 minutos, nos grupos 2 e 3 foram desfeitas as torções dos cordões espermáticos e em seguida os testículos dos animais dos três grupos foram reposicionados no escroto. Após 90 dias foi realizada a orquiectomia bilateral para análise histológica.

**RESULTADOS:** Nos túbulos seminíferos do grupo 1 não se encontrou anormalidades. Nos túbulos seminíferos do Grupo 2, as anormalidades foram 86,6% nos testículos esquerdos e 67,8% nos testículos direitos. Houve diferença estatisticamente significativa quando se compararam os testículos dos grupos 2 e 3.

**CONCLUSÃO:** O propofol minimiza a lesão tecidual em testículos de ratos submetidos à isquemia-reperusão na torção do cordão espermático.

**Descritores:** Propofol. Testículos. Isquemia. Reperusão. Ratos.

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## Introduction

Spermatic cord torsion is a frequent clinical event in emergency care services. It affects children, adolescents, and young adults, prompting emergency surgery because of its potential risk for orchiectomy and infertility, the latter caused primarily by ischemia-reperfusion. Duration and degree of spermatic cord torsion are the foremost factors determining testicular damage<sup>1,2</sup>.

The pathophysiology of spermatic cord torsion initially includes venous occlusion that leads to edema, which in turn causes arterial occlusion and consequent testicular ischemia<sup>3-5</sup>. Ischemia triggers an oxidative mechanism that increases production of reactive oxygen species. This event promotes, among other changes, damage to the cytoskeleton and cell membrane, with dysfunction of calcium pumps, activation of oxidizing enzymes, and mitochondrial changes that further limit the supply of adenosine triphosphate to cells<sup>6</sup>.

Reperfusion occurs subsequent to a period of cellular hypoxia, with increased production and consequent accumulation of purine metabolites—xanthines and hypoxanthines. Hypoxanthine is converted into xanthine, which in turn is transformed into uric acid, generating toxic by-products such as urates, superoxide radicals, and ultimately hydroxyl radicals<sup>6</sup>.

The association of these aggressive components—oxygen free radicals, chemotactic agents and leukocyte aggregation agents—induces lipid peroxidation, with membrane lysis, loss of endothelial integrity, and increased vascular permeability, resulting in tissue damage and cell death. To minimize this tissue damage (which is sometimes reversible), free radicals already formed have to be eliminated and, if possible, prevented from being generated<sup>6,7</sup>.

A number of substances have been investigated for their ability to protect tissues from ischemia-reperfusion effects, including antioxidants such as allopurinol, N-acetylcysteine (NAC), verapamil, vitamins C and E, diltiazem, and propofol.

Since its introduction in the 1980s, propofol (2,6-diisopropylphenol) has been gaining wider acceptance for induction and maintenance of anesthesia and sedation, producing minimal side effects and prompt anesthetic induction. Its chemical structure is similar to that found in the active moieties of endogenous antioxidants such as tocopherol and butylhydroxytoluene. These molecules adhere to cell membranes or to phospholipids to form free radicals, inhibiting lipid peroxidation by reacting with lipid peroxy radicals to form phenoxyl, a non-reactive radical. Propofol might act through a similar mechanism. Its antioxidant effects are thought to result from inhibition of lipid peroxidation by formation

of relatively unreactive free radicals capable of breaking the production chain of radicals that are potentially damaging to cell membranes<sup>8</sup>.

The purpose of this study was to conduct a histological evaluation of the effect of propofol on testicular ischemia-reperfusion in rats.

## Methods

The project was evaluated and approved by the Ethics Committee of the Federal University of Mato Grosso do Sul (UFMS) (permit 226/2009).

Thirty adult male Wistar rats (mean weight: 300g) from the UFMS laboratory animal facilities were used in the experiments. They were given water and chow *ad libitum* and were randomly assigned to one of three groups of 10 animals, as follows: G1: controls; G2: ischemia-reperfusion; G3: ischemia-reperfusion + propofol.

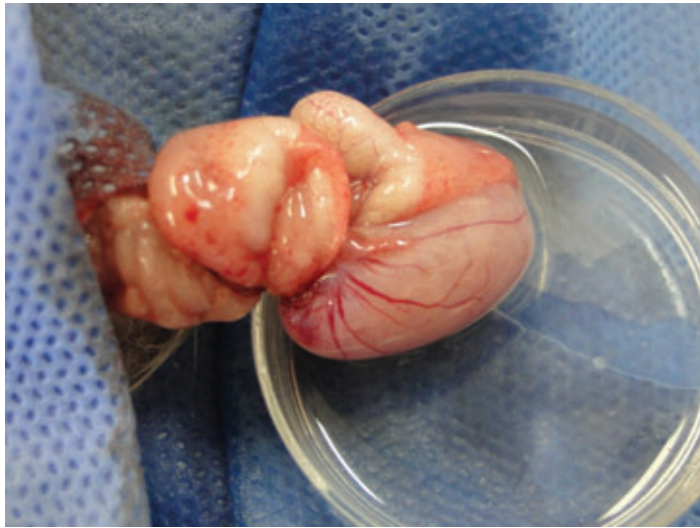
Anesthesia was induced by intramuscular administration of ketamine at 5mg per kilogram of body weight plus xylazine at 2mg per kilogram.

Once anesthesia was achieved, the animals were kept in a supine position and underwent antisepsis of the scrotal region with 2% iodine alcohol.

Using instruments for minor surgical procedures, a longitudinal incision of approximately 2.0cm was made to the scrotal raphe, and tissue planes opened to expose the left testis.

G1 (controls): The left testis was exteriorized, immersed in 0.9% isotonic saline solution for 1h at controlled room temperature, and repositioned in the scrotum. The incision was sutured with catgut 3.0 in continuous stitching on a single plane.

G2 (ischemia-reperfusion): The left testis was exteriorized and its spermatic cord torsioned 720°. The testis was then immersed in 0.9% isotonic saline solution for 1h at controlled room temperature (Figure 1), after which the spermatic cord underwent detorsion and the testis was repositioned in the scrotum. The incision was sutured with catgut 3.0 in continuous stitching on a single plane.



**FIGURE 1** – Torsion of spermatic cord to 720° in groups 2 and 3.

G3 (ischemia-reperfusion + propofol): The left testis was exteriorized and its spermatic cord was torsioned 720°. The testis was immersed in 0.9% isotonic saline solution at controlled room temperature for 1h until detorsion. Fifteen minutes before detorsion, propofol was administered intraperitoneally at 20 mg/kg/h.

At the end of the 1h period the testis was repositioned in the scrotum and the incision was sutured using catgut 3.0 in continuous stitching on a single plane.

After the intervention, the animals were housed in cages and were given oral analgesic (dipyrone) at 15mg/kg every 6h for the first 24h.

The animals were followed up until day 90 post-surgery, at which point bilateral orchietomy was performed (Figure 2).



**FIGURE 2** – Testis and spermatic cord after orchietomy.

All animals were again anesthetized with ketamine plus

xylozine and a longitudinal incision of approximately 2.0cm was made to the scrotal raphe for removal of both testes—a procedure involving cord clamping and hemostasis.

The animals were then euthanized through cardiorespiratory arrest induced by an overdose of ketamine.

The right and left testes were identified, immersed in Bouin’s fluid for 30min, and sent to the UFMS Laboratory of Pathology, where they were kept in a 10% formalin solution for 24h, and embedded in paraffin. Cross-sections 6µm thick were cut using a microtome.

The sections were subjected to hematoxylin-eosin staining and observed under an optical microscope. Histological variables (Table 1) were evaluated according to Cosentino *et al.*<sup>9</sup>.

**TABLE 1** – Histological variables evaluated (according to Cosentino *et al.*<sup>9</sup>).

<b>Seminiferous tubules</b>	
	Loss of sperm cells and spermatids
	Absence of germ cell layers
	Degeneration of germ cell layers
	Structural damage to germ cells
	Tubule rupture
	Reactions in Leydig cells in ruptured tubules
<b>Interstitialium</b>	
	Proliferation of Leydig cells
	Edema
	Hemorrhage
	Granuloma
	Fibrosis

Each testis was graded from 0 to 4 for each variable, with higher grades indicating greater tissue damage in the cross-section examined.

The histological evaluation sought to identify changes in the seminiferous tubules by detecting signs of necrosis and structural damage in the germinal epithelium, as follows:

Grade 0: intact seminiferous tubules

Grade 1: necrosis in superficial cells

Grade 2: necrosis in superficial and deep cells, with structural damage

Grade 3: distal necrosis in germ cells

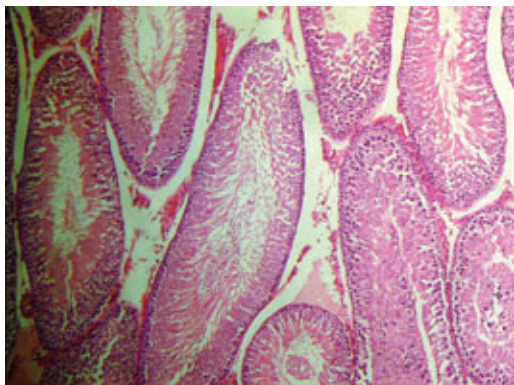
Grade 4: disappearance of tubular structures

Student’s *t*-test ( $p < 0.05$ ) was applied for statistical analysis.

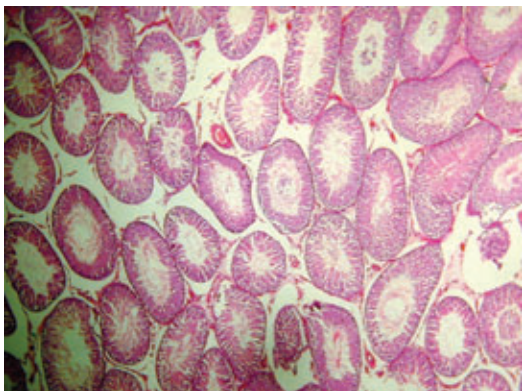


## Results

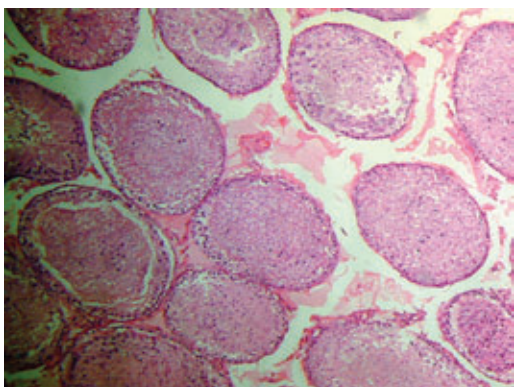
Figures 3-7 depict the histological patterns found for germ cells.



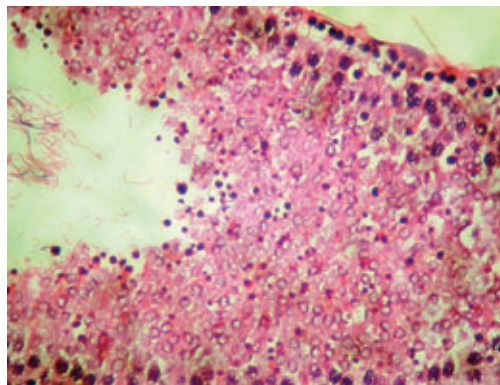
**FIGURE 3** – Photomicrograph of normal seminiferous tubules (G1: controls). Right testis, 100×. Note sperm cell flagella filling the tubules. Germinal epithelium cells are well organized.



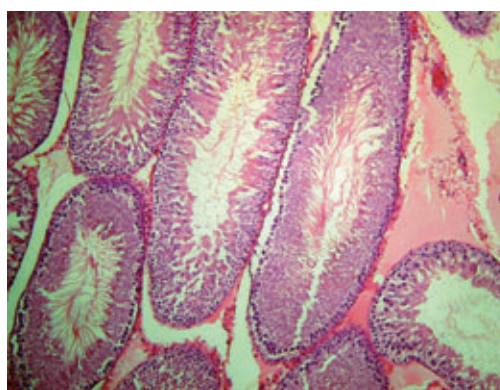
**FIGURE 4** – Photomicrograph of normal seminiferous tubules (G1: controls). Left testis, 40×. Germinal epithelium cells are well organized.



**FIGURE 5** – Photomicrograph of altered seminiferous tubules (G2: ischemia-reperfusion). Left testis, 100×. No viable cells are present. Seminiferous tubule lumen is filled with amorphous material containing cellular debris.



**FIGURE 6** – Photomicrograph of compromised seminiferous tubules (G2: ischemia-reperfusion). Right testis, 400×. Structural damage to epithelium is evident, as well as remnants of sperm cell flagella.



**FIGURE 7** – Photomicrograph of seminiferous tubules (G3: ischemia-reperfusion + propofol). Left testis. Note tubules with normal sperm cell flagella and well-organized germ cells. Partial necrosis and structural damage of the germinal epithelium are evident.

No histological changes related to structural damage were found in G1.

In G2 (Table 2), structural damage to seminiferous tubules was significantly greater in the testis ipsilateral to cord torsion ( $p < 0.05$ ) than in the contralateral testis.

**TABLE 2** – Structural changes in seminiferous tubules, as percentage of abnormality after testicular torsion in rats not treated with propofol.

<b>Group 2 (ischemia-reperfusion)</b>		
Animal	Abnormal seminiferous tubules (%)	
	Left testis	Right testis
1	98	63
2	51	46
3	61	56
4	90	57
5	98	83
6	100	87
7	87	82
8	100	41
9	100	93
10	81	70
Mean	86.6	67.8
SD	17.525854	17.9925911
<i>P</i>		0.00452642

\*Student's *t*-test

In G3 (Table 3), structural damage did not differ significantly between left and right testes.

**TABLE 3** – Structural changes in seminiferous tubules, as percentage of abnormality after testicular torsion in rats treated with propofol.

<b>Group 3 (ischemia-reperfusion + propofol)</b>		
Animal	Abnormal seminiferous tubules (%)	
	Left testis	Right testis
1	59	41
2	63	49
3	34	60
4	39	51
5	63	22
6	56	44
7	61	40
8	89	52
9	78	43
10	31	49
Mean	57.3	45.1
SD	18.5175592	10.0934522
<i>P</i>		0.06866305

\*Student's *t*-test

Table 4 shows that protection against structural damage to seminiferous tubules was significantly higher in G3 (propofol-treated animals) than in G2 (no propofol).

**TABLE 4** – Structural changes to seminiferous tubules, as percentage of abnormality after testicular torsion in rats (group 2 × group 3).

	<b>Group2 (ischemia-reperfusion)</b>		<b>Group3 (ischemia-reperfusion + propofol)</b>		<i>p</i> *
	Mean	SD	Mean	SD	
Left testis	86.6	17.5	57.3	18.5	0.000486
Right testis	67.8	18	45.1	10.1	0.009851

\*Student's *t*-test

## Discussion

Testicular torsion is a frequent occurrence in emergency care services, requiring emergency surgery. Duration and degree of spermatic cord torsion are the two most important factors determining the extent of testicular damage.

A number of substances have been investigated for their potential to protect testicular tissues against the effects of ischemia-reperfusion. In the present study, the antioxidant propofol was employed to prevent testicular damage by eliminating reactive oxygen and nitrogen species and inhibiting lipid peroxidation<sup>10,11</sup>.

Harrison *et al.*<sup>12</sup>, examining the relationship between duration of testicular ischemia-reperfusion and tissue damage in rats, found that testes subjected to 720° torsion for 1h or 2h lost their function, with no improvement 60 days later, despite reestablished blood flow. In the present study, ischemia-reperfusion was maintained for 1h, in line with other studies investigating this phenomenon in rat testes<sup>13,14</sup>.

The literature highlights the positive effects of propofol in preventing tissue damage for such cases. Yagmurduur *et al.*<sup>15</sup> demonstrated that propofol can prevent testicular damage by eliminating reactive oxygen and nitrogen species and inhibiting lipid peroxidation. The present results corroborate these benefits of propofol. Tissue damage was observed in both testes, with greater injury to the organ ipsilateral to cord torsion. In both testes, propofol attenuated the occurrence of tissue damage, indicating the beneficial effects of this compound for testicular ischemia-reperfusion.

The histological classification proposed by Cosentino *et al.*<sup>9</sup> now widely adopted for evaluating testicular tissue injury secondary to testicular ischemia-reperfusion, was used in the present study.

Unsal *et al.*<sup>16</sup> performed 720° testicular torsion with ischemic time of 2h and 25mg/kg/h of propofol. Histological examination revealed well-structured tubules with spaces between cells, in addition to some areas of hemorrhage. Similar results were found in the present study, despite shorter ischemic time (1h) and propofol administration at 20mg/kg/h.

Good results were also obtained by Avlan *et al.*<sup>17</sup>, whose study involved 720° torsion and ischemic time of 4h, administration of selenium, and histological evaluation based on Cosentino *et al.*<sup>9</sup>.

Silva *et al.*<sup>18</sup> also performed spermatic cord torsion at 720° for 1h, administering allopurinol intraperitoneally 45min after torsion. Allopurinol, however, failed to protect testicular tissues against the effects of ischemia-reperfusion, in contrast to

the results obtained by the present study using propofol.

Erol *et al.*<sup>19</sup>, investigating spermatic cord torsion in rats, used the intraperitoneal route to inject the anesthetic. Torsion to 720° was maintained for 1h. Vardenafil was administered to protect against ischemia-reperfusion, reducing the occurrence of necrosis.

Yurtçu *et al.*<sup>20</sup> performed 720° torsion of spermatic cord for 6h and administered melatonin before detorsion. Histological evaluation, conducted according to Cosentino *et al.*<sup>9</sup>, revealed a good level of protection.

Based on the results of this study and those in the literature, it can be inferred that administration of propofol at the dose investigated conferred protection to testes of rats against ischemia-reperfusion.

## Conclusion

Propofol conferred tissue protection against the effects of testicular ischemia-reperfusion in rats.

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