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Testicular changes in adult dogs undergoing chemotherapy treatment with vincristine sulfate

[Alterações testiculares de cães adultos submetidos ao tratamento quimioterápico por sulfato de vincristina]

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Abstract: Vincristine sulfate is a first-line antineoplastic drug for the treatment of canine transmissible venereal tumor (tvCT). However, it can cause adverse effects such as anorexia and myelosuppression. In humans, its use is associated with gonadal disorders, affecting fertility. In dogs, it can cause testicular degeneration and impact sperm quality and count. The aim of this study was to evaluate the effects of vincristine sulfate on the testicular parenchyma of dogs, encompassing the potential effects and extent of damage caused to spermatogenesis. Six control animals and six treated with vincristine sulfate were used. After the end of chemotherapy, the treated animals underwent orchiectomy, at which time biometric measurements (body weight and testicular weights, axes, and volumes) were performed. Testicular samples were collected, fixed, and processed for analysis of testicular morphometric measurements (tubular diameter and lumen, major and minor tubular axes, seminiferous epithelium height, and tubular lumen area) and stereological measurements (tubular volumes and volume densities of tubular and interstitial testicular tissue). Stereology was performed on 200 seminiferous tubules. Administration of vincristine sulfate in dogs resulted in a reduction of the morphometric and stereological variables epithelial height, lumen diameter, tubular lumen area, and seminiferous tubule volume; in addition to structural disorganization and intraepithelial vacuoles. It is concluded that vincristine sulfate can cause alterations in the testicular parenchyma of dogs undergoing chemotherapy treatment with this drug.

Keywords: seminiferous epithelium; male gonads; morphology; oncology; chemotherapy.

Resumo: O sulfato de vincristina é um fármaco antineoplásico de primeira escolha para o tratamento do tumor venéreo transmissível canino (TVTc). No entanto, pode causar efeitos adversos como anorexia e mielossupressão. Em humanos, sua utilização está associada a distúrbios gonadais, afetando a fertilidade. Em cães, pode causar degeneração testicular e impactar na qualidade e contagem de espermatozoides. Objetivou-se avaliar os efeitos do sulfato de vincristina sobre o parênquima testicular de cães, abrangendo os potenciais efeitos e a extensão de danos causados à espermatogênese. Foram utilizados seis animais controle e seis tratados com sulfato de vincristina, na dose 0,6 mg/m2 a cada sete dias. Após o término da quimioterapia, os animais tratados foram submetidos a orquiectomia, momento em que foram realizadas medidas biométricas (peso corpóreo, testiculares, eixos e volumes testiculares). Amostras de testículos foram coletadas, fixadas e

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processadas para análise das medidas morfométricas testiculares (diâmetro e lúmen tubulares, eixos tubulares maiores e menores, altura do epitélio seminífero e área do lúmen tubular) e estereológicas (volumes tubulares e as densidades de volume do tecido tubular e do tecido intersticial testicular). A estereologia foi realizada em 200 túbulos seminíferos. A administração do sulfato de vincristina em cães resultou na redução das variáveis morfométricas e estereológicas altura do epitélio, diâmetro do lúmen, área do lúmen tubular e volume dos túbulos seminíferos; além de desorganização estrutural e vacúolos intraepiteliais. Conclui-se que o sulfato de vincristina pode provocar alterações no parênquima testicular de cães submetidos a quimioterapia com esse fármaco.

Palavras-chave: epitélio seminífero; gônadas masculinas; morfologia; oncologia; quimioterapia.

1. Introduction

The use of antineoplastic agents has become increasingly frequent in both human and veterinary medicine, acting primarily through the inhibition of DNA and RNA synthesis and cellular replication, to control the proliferation of neoplastic cells. However, these cytotoxic agents also affect normal cells with high proliferative rates, such as those in the bone marrow, epithelial tissues, gonads, and hair follicles [1, 2].

Vincristine sulfate, a vinca alkaloid derived from *Vinca rosea*, exerts its antimitotic effect by inhibiting microtubule polymerization, leading to cell cycle arrest at metaphase. It is widely used in veterinary medicine for the treatment of neoplasms and is considered the agent of choice for canine transmissible venereal tumor (CTVT) due to high remission rates associated with it. Additionally, it is extensively used in human medicine for the treatment of various malignant neoplasms, including lung cancer, acute lymphoblastic leukemia, Hodgkin's disease, acute myeloid leukemia, and neuroblastoma [7]. Despite its therapeutic efficacy, vincristine sulfate is associated with systemic adverse effects and potential gonadal alterations [2].

The effects of antineoplastic agents and the long-term consequences of chemotherapy in humans are well documented, particularly regarding gonadal dysfunction in both sexes, which is associated with the toxicity of vincristine-containing regimens [9, 10]. Antineoplastic agents damage the germinal epithelium, resulting in oligozoospermia or azoospermia in humans and impaired fertility in experimental animal models, including rats [17–20]. In dogs, it induces alterations in the testicular parenchyma, including vacuolization, epithelial desquamation, and reduced sperm quality due to its direct effects on the epididymis and vas deferens [21, 22].

In this context, morphological and stereological analyses of testicular tissue represent robust approaches for assessing reproductive function, allowing the identification of structural alterations associated with spermatogenic activity [24-26].

Therefore, this study aimed at investigating and characterizing morphological and stereological changes in the testicular parenchyma, including the potential effects and extent of damage to spermatogenesis following antineoplastic chemotherapy with vincristine sulfate. Furthermore, this study evaluated the effects of vincristine sulfate on fertility and reproductive health in dogs undergoing treatment for canine transmissible venereal tumor (CTVT), considering possible chemotherapy-induced testicular alterations. The findings contribute to the refinement of therapeutic protocols aimed at minimizing reproductive impacts and providing insights into the gonadal effects of antineoplastic chemotherapeutic agents in comparative studies across species, including humans.

2. Material and methods

The study was conducted in accordance with ethical standards and was approved by the Ethics and Research Committee of the Federal University of Campina Grande (UFCG), under protocol no. 13/2024, at the Center for Rural Health and Technology (CSTR), Patos, PB, Brazil.

2.1 Animals

Twelve intact mixed-breed dogs aged between two and three years were selected. Of these, six were clinically healthy, and six were diagnosed with canine transmissible venereal tumor (CTVT). The animals were included in the study upon owner consent, in accordance with the Informed Consent Form.

2.2 Experimental design

The dogs were allocated into two experimental groups: a control group (CG) and a vincristine-treated group (VG), each consisting of six animals (n = 12). The CG comprised clinically healthy dogs, not affected by CTVT, recruited from routine clinical care at the University Veterinary Hospital. They underwent elective orchiectomy and were aged between two and three years, with body weights ranging from 16 to 23 kg. All of them were considered clinically stable and had no history of undergoing chemotherapy treatment. In the CG, clinical evaluations, hematological tests, and serum biochemical analyses were performed before the surgical procedure, following the hospital's standard preoperative protocol. No placebo was administered, and they served as a comparative reference for the evaluation of testicular alterations observed in the VG.

In the VG, animals of the same age range, unneutered, mixed breed, with body weights ranging from 7.5 to 24 kg, and diagnosed with CTVT were selected. They received vincristine sulfate intravenously at a dose of 0.6 mg/m² [3] , administered every seven days, for a minimum of four and a maximum of seven sessions. Orchiectomy was performed after clinical confirmations of tumor remission, immediately following completion of the individual chemotherapy protocol for each animal.

Clinical and laboratory evaluations were performed before the initiation of chemotherapy and throughout the treatment period. Complete blood counts were obtained before each vincristine administration to monitor potential hematological alterations associated with chemotherapy. Serum biochemical analyses, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine, were performed every 15 days during the chemotherapy protocol.

2.3 Sample collection and processing

Following orchiectomy, the testes were immediately dissected and macroscopically evaluated for integrity, color, and general appearance. Subsequently, biometric assessment of the organs was performed, including absolute testicular weight, relative testicular weight, measurements of the major and minor axes, and calculation of testicular volume, as described in section 2.4.

The testes were then transversely sectioned by hemisection, encompassing the proximal and distal poles as well as the intermediate region, to ensure adequate fixative penetration and preservation of internal structures. The samples obtained were fixed by immersion in Bouin's solution for a total period of 24 hours. After fixation, the tissue fragments were washed under running water to remove excess fixative and subsequently transferred to 70 % ethanol, where they were stored until histological processing.

2.4 Biometry, morphometry, and stereology

Testicular biometry was performed immediately after orchiectomy and before histological processing of the samples. The animals were weighed to determine total body weight, and absolute testicular weight (in grams) was measured using a semi-analytical balance (AD500 Marte®). Relative testicular weight was calculated using the formula (absolute testicular weight × 100 g/body weight), as described by Brilhante et al. [19]. In addition, the major and minor testicular axes (in millimeters) were measured using a digital caliper. Testicular volume was determined according to the method described by Scherle [40]. Total testicular volume was calculated using the formula: volume = density × mass, where specific gravity is defined as the ratio between the density of the evaluated organ and the density of the reference substance (water) [31].

The mean total testicular volume for each group was obtained by calculating the arithmetic mean of the volumes of both testes from each animal within their respective control and experimental groups. Morphometric analysis was performed using the Image-Pro Express 6.0 software on a computer coupled to an Olympus BX40 binocular light microscope with a 10× objective. A total of 200 seminiferous tubules per animal were randomly measured. Twenty-five seminiferous tubules were evaluated per testicular section through complete and sequential scanning. The following parameters were measured: diameter of the seminiferous tubule, diameter of the tubular lumen, height of the seminiferous epithelium, tubular area, lumen area, and minor axis of the seminiferous tubule. All variables were measured individually, and subsequently, weighted means were calculated for each group.

Stereological analysis was performed microscopically on sections of both the right and left testes. The volume density (Vv) of the tubular and interstitial compartments of the testicular parenchyma was evaluated. Stereological variables were obtained by point counting using systematic random sampling of images acquired with Image-Pro Express 6.0 on a computer coupled to an Olympus BX40 trinocular light microscope at 10× magnification, followed by point counting using the ImageJ software. A grid of 190 points was applied, totaling 16,000 points per testis.

The densities of interstitial tissue and seminiferous tubules (mm^3) were calculated based on the percentage obtained from the volume density (Vv) of each tissue, according to Miraglia and Hayashi [41] . The formula used for volume density was: $V\% = \Sigma P\text{ (structure)}/\Sigma P\text{ (tissue)} \times 100$, where V% represents the final volume percentage, ΣP (structure) is the sum of points falling on a given structure, and ΣP (tissue) is the total number of points falling on the visible tissue.

Mean tubular and interstitial volumes per animal were also calculated using data obtained from the total volume of the right and left testes. The formula used to calculate volume was: $Vv = T \times a/p \times \Sigma Pi$, where T is section thickness, a/p is the area associated with each point, and ΣPi is the total number of counted points.

Biometric, morphometric, and stereological analyses were not performed in a blinded manner, as the researcher responsible for the measurements was aware of the allocation of animals into experimental groups due to clinical follow-up and the therapeutic protocol applied.

2.5 Statistical analysis

Data were assessed for normality using the Shapiro–Wilk test. When $p > 0.05$, data were considered to follow a normal (parametric) distribution, whereas $p < 0.05$ indicated a non-parametric distribution. For parametric data, Student's *t*-test was applied. Data that did not meet the assumptions of normality and homogeneity of variance were analyzed using the non-parametric

Mann–Whitney *U* test. Identical letters were used to indicate non-significant differences ($p > 0.05$), whereas different letters indicated statistically significant differences ($p \leq 0.05$). Statistical analyses were performed using the BioEstat 5.0 software.

3. Results and discussion

Assessment of the health history and pre-procedural anamnesis of each animal was performed as a standard protocol. Those in the control group (CG) exhibited histological characteristics within normal limits for the species (Figure 1). The biometric variables, including body weight, absolute and relative testicular weight, total testicular volume, and major and minor testicular axes, are presented in Table 1.

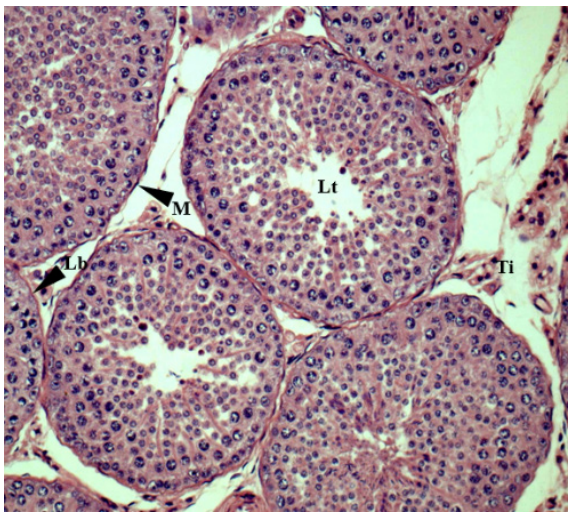

Figure 1. Photomicrograph of the testis from a dog in the control group (CG). Hematoxylin and eosin (H&E) staining, performed at the Animal Pathology Laboratory of the Prof. Dr. Ivon Macêdo Tabosa Veterinary Hospital, Federal University of Campina Grande (UFCG), Patos, PB, Brazil. Magnification: 20×. Typical testicular architecture is observed, with well-organized seminiferous tubules, a structured germinal epithelium, evident tubular lumen (Lt), intact basement membrane (Lb), organized myoid cells (M), and preserved interstitial tissue (Ti).

Table 1. Biometric variables (mean ± standard deviation) in control (CG) and vincristine-treated (VG) dogs attended at the Prof. Dr. Ivon Macêdo Tabosa Veterinary Hospital, Federal University of Campina Grande (UFCG).

	BIOMETRIC VARIABLES					
GROUPS	**BW (kg)**	**ATW (g)**	**RTW (%)**	**ETMA (mm)**	**ETME (mm)**	**TTV (mm³)**
CG	19.71±2.80[a]	12.0±2.35[a]	0.05±0.02[a]	31.35±6.79[a]	23.10±5.92[a]	11.57±2.26[a]
VG	16.48±5.46[a]	10.93±3.25[a]	0.06±0.01[a]	28.47±6.43[a]	22.87±5.37[a]	10.48±3.14[a]

Body weight (BW), absolute testicular weight (ATW), relative testicular weight (RTW), major and minor testicular axes mTA), and total testicular volume (TTV). Means followed by different letters differ significantly according to Student's *t*-test ($p \leq 0.05$).

The mean body weight of the vincristine-treated group (VG) did not differ significantly ($p > 0.05$) from that of the CG. In the present study, animals in the VG showed smaller fluctuations in body weight and absolute testicular weight during treatment, when compared to the CG.

A study by Veloso et al. [27] also reported no significant changes in the body weight of rats treated with vincristine sulfate and euthanized at 64 and 127 days of age. The authors suggested that, although the drug inhibits the renewal of the gastrointestinal epithelium, recovery of the digestive system likely occurred between chemotherapy administration and the time of euthanasia.

Other studies have reported body weight fluctuations in rats treated not only with vincristine sulfate but also with other antineoplastic agents [28, 29].

One hypothesis for the observed body weight fluctuations in the VG may be related to the systemic toxicity of vincristine, which can induce anorexia and nausea. However, in the present study, it was observed that the selected dogs exhibited variability in body size, which may have influenced both body weight and testicular weight, given the direct relationship between these variables [30, 31].

Absolute and relative testicular weights in the vincristine-treated group (VG) did not differ significantly ($p > 0.05$) from those of the control group (CG). Lirdi et al. [32] also reported no variation in absolute and relative testicular weights in rats treated with cisplatin, which was likely attributed to lymphatic and/or interstitial edema. In the study by Jagetia et al. [33], the use of vinblastine at doses ranging from 0.01 to 0.5 mg/kg did not result in changes in testicular weight; however, increasing the dose led to a decline in this variable after 14 days. In contrast, Veloso et al. [20] reported significant reductions in absolute and relative testicular weights in rats treated with vincristine sulfate, attributing these findings to the drug dosage.

The cumulative effects of vinca alkaloids, as demonstrated by Jagetia et al. [33], particularly at higher doses, should not be disregarded when considering the potential for recovery of testicular weight, especially in relation to the interval between the last chemotherapy administration and the time of orchiectomy. Silva et al. [34] reported that alterations in the testicular parenchyma depend on the type of antineoplastic agent, dosage regimen (single or fractionated), and the age of the patient.

The major and minor testicular axes in the VG did not differ significantly ($p > 0.05$) from those in the CG, in agreement with Gobello and Corrada [35], who reported that dogs treated with vincristine sulfate at a dose of 0.7 mg/m² showed no changes in these variables. In the present study, a possible compensatory increase in interstitial volume may have contributed to the maintenance of testicular axes. Sales [36] stated that changes in the size of the major and minor axes (diameter) are primarily related to total testicular volume.

Similarly, the VG showed no significant difference ($p > 0.05$) in total testicular volume compared to the CG, suggesting a compensatory balance among the structural components of the testes. Supporting the findings of Amann and Schanbacher [37], the absence of changes in total testicular volume does not necessarily indicate the absence of deleterious effects on germ cells, since total testicular volume results from the sum of seminiferous tubule volume and interstitial tissue volume, both of which are subject to variation.

Exposure to antineoplastic agents may increase interstitial volume, such that the loss of germ cells is not necessarily associated with a reduction in testicular volume or related variables. Maintenance of testicular volume may result from expansion of the intertubular compartment, compensating for germ cell loss and influencing organ weight and testicular axes [29,32], thereby explaining the preservation of major and minor testicular axes.

Biometric parameters showed no significant alterations in the treated group, corroborating studies in sexually mature rats treated with doxorubicin, which reported no differences in body weight, absolute and relative testicular weights, or total testicular volume compared to the CG [34, 38].

The results of the morphometric variables, including diameter of the seminiferous tubule, height of the seminiferous epithelium, diameter of the tubular lumen, minor axis of the seminiferous tubule, area of the seminiferous tubule, and area of the lumen, are presented in Table 2.

Table 2. Morphometric variables (mean ± standard deviation) in control (CG) and vincristine-treated (VG) dogs attended at the Prof. Dr. Ivon Macêdo Tabosa Veterinary Hospital, Federal University of Campina Grande (UFCG).

	MORPHOMETRIC VARIABLES					
GROUPS	**STD (µm)**	**SEH (µm)**	**TLD (µm)**	**mAST (µm²)**	**STA (µm²)**	**TLA (µm²)**
CG	192.92±8.67[a]	72.69±3.93[a]	58.54±7.92[a]	184.53±5.64[a]	634.04±19.42[a]	213.26±28.89[a]
VG	190.88±9.82[a]	30.14±5.58[b]	138.82±21.60[b]	183.84±7.34 [a]	647.79±42.42[a]	466.99±64.73[b]

Seminiferous tubule diameter (STD), seminiferous epithelium height (SEH), tubular lumen diameter (TLD), minor axis of the seminiferous tubule (mAST), seminiferous tubule area (STA), and tubular lumen area (TLA) in testes of control (CG) and vincristine-treated (VG) dogs. Different letters indicate statistically significant differences according to Student's *t*-test ($p \leq 0.05$), whereas identical letters indicate no statistically significant differences ($p > 0.05$).

The mean diameter of the seminiferous tubule did not differ significantly ($p > 0.05$) compared to the CG. Similarly, Martins [39], in a study with rats treated with 5-fluorouracil, did not observe a reduction in tubular diameter, although a decrease in the height of the seminiferous epithelium and a notable degree of tubular degeneration were identified—findings consistent with those observed in the present study.

In contrast, Silva et al. [34] reported a reduction in tubular diameter in rats treated with doxorubicin, which was attributed to massive germ cell loss. A similar alteration was described by Veloso et al. [20], who reported seminiferous tubule atrophy resulting from the deleterious effects of vincristine sulfate.

Considering that tubular diameter may remain unchanged despite alterations in variables such as epithelial height and lumen area, one possible explanation is that vincristine sulfate in dogs may primarily affect seminiferous epithelium cells without necessarily altering tubular diameter or minor axis, likely due to the absence of marked tubular atrophy.

Based on the variations in tubular size observed in this study, França and Russell [31] reported that the number and dimensions of seminiferous tubules may vary among breeds within the same species, typically ranging from 180 to 300 µm in adult mammals.

Seminiferous epithelium height differed significantly from the control group ($p \leq 0.05$), showing a reduction of 58.53 % in the treated group. This effect is attributed to the action of vincristine sulfate on the seminiferous epithelium, likely due to disruption of spermatogonial cell proliferation and subsequent cell detachment. Veloso et al. [20] support these findings, describing the effects of vincristine sulfate on rats, particularly on seminiferous tubule cells, with deleterious effects primarily affecting type A spermatogonia. Furthermore, the absence of epithelial recovery over time suggests the possibility of permanent damage.

In an *in vitro* study, vincristine sulfate was shown to induce cell cycle arrest and germ cell death without causing genotoxic effects, reducing cell viability and activating apoptosis in a dose- and time-dependent manner [40]. It is well established that most antineoplastic agents target the division of male germ cells [17], with proliferating diploid cells representing the primary targets of this class of drugs, which explains the high sensitivity of germ cells [41].

Studies involving antineoplastic agents such as adriamycin, 1-β-D-arabinofuranosylcytosine, bleomycin, cyclophosphamide, hydroxyurea, vinblastine, vincristine sulfate, and doxorubicin have demonstrated that these drugs affect germ cells, particularly type A spermatogonia, as well

as reduce intermediate spermatogonia, type B spermatogonia, primary spermatocytes, round spermatids, and Sertoli cells, ultimately resulting in depletion of the seminiferous epithelium [42–44]. Damage to the seminiferous epithelium in animals treated with vincristine sulfate may occur early during treatment and persist chronically in adult animals [45].

The tubular lumen diameter showed a significant increase compared to the control group, with an increase of 137.13 %. This finding resulted from reduced cellular density of the seminiferous epithelium. Consequently, the tubular lumen area also increased by 118.97 %, differing significantly from the control group ($p \leq 0.05$), reflecting cellular depletion induced by the effects of vincristine sulfate, in agreement with the findings of Veloso et al. [20] and Lucena [45].

Variables related to the minor axis of the seminiferous tubule and tubular area did not differ significantly ($p > 0.05$). Nasrabadi, Mohammadnejad, and Nikpour [46] reported that alterations in testicular axes (major and minor) are associated with a seminiferous tubule atrophy caused by the deleterious effects of vincristine sulfate. In the present study, one possible explanation is that vincristine sulfate selectively affects germ cells while sparing other structural components. It was observed that tubular areas and axes were less sensitive to vincristine-induced changes compared to other testicular morphological parameters, particularly germ cells, which showed greater impairment, leading to reduction of the seminiferous epithelium and enlargement of the tubular lumen.

It is essential to determine whether deleterious effects occur through direct or indirect actions on Sertoli cells during development, or whether they result from direct or indirect effects on germ cells. In most studies, conducted in prepubertal rats receiving a single dose of a chemotherapeutic agent, the mechanisms underlying germ cell damage may differ from those observed in adult animals, which may explain the differences in the variables analyzed.

The morphological alterations observed in the testes of the VG included cellular depletion, loss of the structural organization of germ cell layers, desquamation of the seminiferous epithelium with the presence of cellular debris within the lumen (Figure 2), and intraepithelial vacuolization (Figure 3). These findings were more severe in tubules with greater epithelial damage. The observed vacuolization may represent spaces left by germ cells that underwent apoptosis or degeneration, or resulted from detachment of these cells into the tubular lumen [45].

These findings are consistent with studies conducted in rats treated with antineoplastic agents such as doxorubicin and vincristine sulfate [19, 20, 45, 47], as well as in dogs, as reported by Daleck et al. [21] using vincristine sulfate. These alterations are directly related to the cytotoxic effects of vincristine sulfate on seminiferous epithelium cells [20].

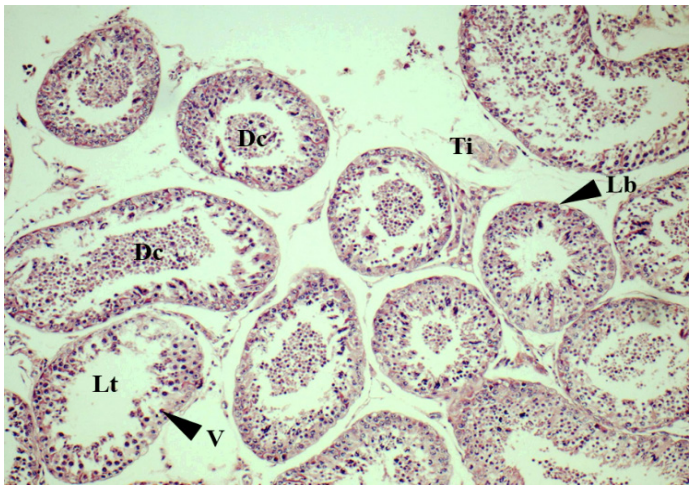

Figure 2. Photomicrograph of the testis from a dog in the vincristine-treated group (VG). Hematoxylin and eosin (H&E) staining, performed at the Animal Pathology Laboratory of the Prof. Dr. Ivon Macêdo Tabosa Veterinary Hospital, Federal University of Campina Grande (UFCG), Patos, PB, Brazil. Magnification: 10×. Scale bar = 50 µm. Disorganization of the seminiferous epithelium, cellular desquamation (Dc), and intraepithelial vacuoles (V) are observed. Tubular lumen (Lt), basement membrane (Lb), and interstitial tissue (Ti) are also indicated.

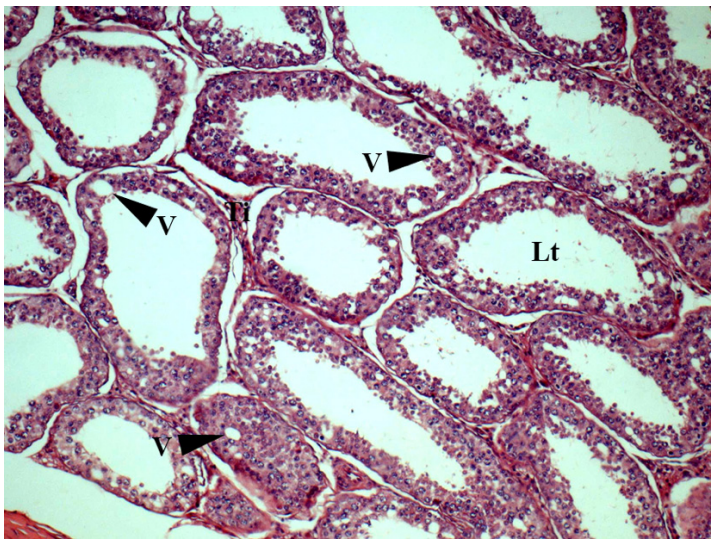

Figure 3. Photomicrograph of the testis from dogs in the vincristine-treated group (VG). Hematoxylin and eosin (H&E) staining, performed at the Animal Pathology Laboratory of the Prof. Dr. Ivon Macêdo Tabosa Veterinary Hospital, Federal University of Campina Grande (UFCG), Patos, PB, Brazil. Magnification: 10×. Disorganization and vacuolization (V) are observed in the testicular parenchyma. Tubular lumen (Lt) and interstitial tissue (Ti) are indicated.

Cellular desquamation is suggested to result from the loss of cytoplasmic connections between germ cells and Sertoli cells within the seminiferous epithelium, leading to reduced intercellular communication [48].

The present study demonstrated a statistically significant difference ($p \leq 0.05$) in the volume density of seminiferous tubules in the VG compared to the CG, with a reduction of 44.61 %, as shown in Table 3. This finding indicates a decrease in the number of seminiferous tubule profiles per unit area, suggesting impairment of the testicular parenchyma and a reduction in the germ cell population, corroborating the findings of Veloso et al. [27].

Table 3. Volume density of seminiferous tubules (VvST), volume density of interstitial tissue (VvIT), seminiferous tubule volume (STV), and interstitial tissue volume (ITV) (mean ± standard deviation) in testes of control (CG) and vincristine-treated (VG) dogs attended at the Prof. Dr. Ivon Macêdo Tabosa Veterinary Hospital, Federal University of Campina Grande (UFCG).

	STEREOLOGICAL VARIABLES			
GROUPS	**VvST (%)**	**VvIT (%)**	**STV (mm³)**	**ITV (mm³)**
CG	63,21±2,40[a]	36,79±2,40[a]	6,45±0,24[a]	4,95±0,24[a]
VG	35,01±5,54[b]	64,99±5,54[b]	3,75±0,56[b]	6,63±0,56[b]

Different letters indicate statistically significant differences according to Student's *t*-test ($p \leq 0.05$), whereas identical letters indicate no statistically significant differences ($p > 0.05$).

The volume density of interstitial testicular tissue (Table 3) in the VG also differed significantly from that of the CG ($p \leq 0.05$), showing an increase of 76.65 %. This may represent a compensatory response between the interstitial tissue and the testicular parenchyma, as both constitute the main structural components of the testes. It was observed that the volume density of seminiferous tubules and that of interstitial tissue exhibited an inverse relationship, as described by other authors [27, 34, 36].

Data regarding the volume of seminiferous tubules in the treated group (Table 3) also differed significantly from the control group, showing a reduction of 41.86 %. In the study by Brilhante et al. [19], administration of doxorubicin resulted in a decrease in testicular volume as a consequence of germ cell death. As these agents directly or indirectly affect the seminiferous epithelium, it is suggested that the observed reduction is attributable to cellular depletion.

Conversely, the volume of the testicular interstitium (Table 3) increased by 33.94 % compared to the control group ($p \leq 0.05$), suggesting a compensatory reduction of the seminiferous tubules, which resulted in increased spacing between tubular profiles. These findings are consistent with those reported by Veloso et al. [27], who described dispersion of seminiferous tubules in treated animals.

4. Conclusion

Administration of therapeutic doses of vincristine sulfate in adult dogs induces structural alterations in the seminiferous epithelium, with consequent effects on germ cells. However, functional reproductive parameters were not evaluated, and therefore, it is not possible to infer from the present data whether reproductive capacity is impaired. Further studies are required to determine whether the observed alterations are reversible and to assess their impact on reproductive function.

Conflict of interest statement

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Data availability statement

The complete dataset supporting the findings of this study is available from the corresponding author upon reasonable request.

Author contributions

Conceptualization: Reis, T. S. Formal analysis: Reis, T. S. Research: Nascimento, B.H. R.; Freitas, A. B. A. A.; Reis, T. S. Data curation: Reis, T. S. Data analysis: Reis, T. S. Drafting (original outline): Reis, T. S.; Toledo, G. N. Writing: Reis, T. S. Project administration: Toledo, G. N. Resources: Brilhante, O. S.; Araújo, P. P. B.

Generative AI use statement

The authors did not use generative artificial intelligence tools or technologies in the creation or editing of any part of this manuscript.

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