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Histological Typing and Morphological Characterization of Canine Seminomas

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Abstract: The aim of this study was to evaluate seminomas based on cell morphology and histological structures and classify them into subtypes. Thirty canine seminomas were stained with hematoxylin-eosin (HE) and Periodic Acid Schiff (PAS). PAS-positive cases were classified as Classical seminoma (CS) (n:5), while PAS-negative cases were categorized as Spermatocytic seminoma (SS) (n:23). Cases exhibiting both positive and negative staining were evaluated as Spermatocytic/Classical seminoma (n:2). Carcinoma in situ were observed in the cases with Classical seminoma (n:3). The cell morphology and mitotic indices of each case were given in detail. Canine seminomas were classified into three subtypes: intratubular (n:12), diffuse (n:1), and intratubular/diffuse (n:10). As a result, it was thought that SS and CS types could be observed together, and it was observed that the intratubular/diffuse subtype is a third type characterized by its transformation from intratubular to diffuse form.

Keywords: *Canine seminoma, Classical seminoma, Histopathology, Spermatocytic seminoma.*

Köpek seminomlarının histolojik tiplendirilmesi ve morfolojik karakterizasyonu

Özet: Çalışmanın amacını, seminomları hücre morfolojisi ve histolojik yapılarına göre değerlendirerek alt tiplerine ayırmak oluşturdu. Otuz adet seminom hematoksilen-eozin (HE) ve Periyodik Asit Schiff (PAS) ile boyandı. PAS pozitif vakalar Klasik seminom (CS) (n:5), PAS negatif vakalar ise Spermatositik seminom (SS) (n:23) olarak sınıflandırıldı. Hem pozitif hem de negatif boyanma gösteren vakalar Spermatositik/Klasik seminom (n:2) olarak değerlendirildi. Klasik seminom vakalarında Carcinoma in situ gözlendi (n:3). Her bir vakaya ait hücre morfolojisi ve mitotik indeksleri ayrıntılı olarak verildi. Köpek seminomları üç alt tipe ayrıldı: intratübüler (n:12), diffüz (n:1) ve intratübüler/diffüz (n:10). Sonuç olarak, SS ve CS tiplerinin bir arada görülebileceği düşünüldü ve intratübülerden diffüz forma dönüşümüyle karakterize edilen üçüncü bir tipin intratübüler/diffüz alt türü olduğu gözlendi.

Anahtar Kelimeler: *Histopatoloji, Klasik seminom, Köpek seminomları, Spermatositik seminoma.*

Introduction

Seminomas in dogs are the most frequently encountered tumors in testicular germ cell tumors, originating from germ cells within the seminiferous tubules. While they are more commonly observed in older dogs, certain breeds such as Boxers, German Shepherds, Maltese, and Norwegian Elkhounds are reported as predisposed. Cryptorchidism is also highlighted as a significant predisposing factor in the development of seminomas (Agnew and MacLachlan, 2016; Hernández-Jardón et al., 2022).

In humans, seminomas are classified into two subtypes according to the WHO: Classical Seminoma (CS) and Spermatocytic Seminoma (SS) (Mostofy and Sesterhenn,1998). Classical Seminoma, also called Typical Seminoma, is observed in young men and clinically described as aggressive and malignant. On the other hand, the second subtype, Spermatocytic Seminoma, is less malignant and more commonly observed in older men (Agnew and MacLachlan, 2016; Grieco et al., 2007; Mostofy and Sesterhenn,1998).

The difference in biological behaviour is due to the differences in the cells from which they originate. CS originates from gonocytes, while SS originates from mature spermatocytes. Gonocytes are progenitor cells that play a role in the development of prespermatogonia and spermatogonia, and they stain positively with periodic acid-Schiff (PAS) due to the presence of glycogen. For distinguishing between the two types, PAS staining and immunohistochemical expression of placental alkaline phosphatase (PLAP) can be used (Agnew and MacLachlan, 2016; Grieco et al., 2007).

The initial stage of CS, considered as a preneoplastic lesion or referred to as "carcinoma in situ (CIS)," consists of embryonic morphology composed of PAS and PLAP-positive germ cells. As a result, CIS structures are not observed in SS. In human medicine, although the CIS structure is typically observed in CS, it is believed to be the precursor of most germ tumors (Agnew and MacLachlan, 2016; Grieco et al., 2007; Skakkebaek et al., 1987).

In domestic animals, seminomas are histomorphologically categorized into two forms: intratubular and diffuse. If neoplastic germ cells are located within the seminiferous tubules, it is classified as intratubular; if they are spread throughout the entire field independent of tubules, they are considered diffuse. Canine seminomas are morphologically more compatible with SS. SS exhibit neoplastic germ cells with varying sizes, indistinct borders, amphophilic cytoplasm, vesicular nuclei, and prominent nucleoli. Giant cells and mitotic figures are frequently observed in the SS type. Due to the filamentous chromatin appearance, typical "spiremes" mitosis is commonly encountered in the SS type. Despite being intratubular, CIS should be distinguished with the help of PAS-positive staining and cellular morphology from Spermatocytic Seminoma (SS). On the other hand, Classical Seminoma (CS) cells are of a single type and are monomorphic. They have abundant cytoplasm with distinct borders and show PAS and PLAP-positive staining (Agnew and MacLachlan, 2016; Grieco et al., 2007; Kennedy et al., 1998).

The comprehensive aim of the study was to evaluate canine seminomas. In the study, the investigation and comparison of 30 cases of seminoma were conducted, considering the existing classifications and subtyping methods.

Materials and Methods

This study is not subject to HADYEK permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

A total of 30 testicular tissues diagnosed as seminoma at Ankara University Veterinary Faculty Pathology Department were included in the study. Among these, 27 were operation materials and 3 were obtained post-mortem (necropsy material). Paraffin-embedded blocks of the testicles were cut at a thickness of 4-5 µm using a microtome, and after routine processes, they were stained with hematoxylin-eosin (HE) and Periodic Acid Schiff (PAS). Based on PAS staining, those that stained positive were classified as “Classical Seminoma (CS)”, while negative staining indicated “Spermatocytic Seminoma (SS)”. In cases where both negative and positive staining were observed in certain proportions, a modified subtyping was used as “Classical/Spermatocytic Seminoma (CS/SS)”. Cellular structures were examined in detail. For this purpose, the morphological variation in tumor cells (monomorphic/polymorphic), the clarity of their boundaries (indistinct border/abundant border), and the cytoplasmic characteristics (abundant cytoplasm/amphophilic less abundant cytoplasm) were examined. The presence of giant cells and spiremes mitosis were noted. The mitotic index was assessed by counting the mitotic figures present in 10 fields (x400). If the total number of mitotic figures in 10 random fields was less than 10, it was scored as “1”; between 10 and 50, it was scored as “2”; and if greater than 50, it was scored as “3”. As for histological patterns, they were classified according to the animal World Health Organization (WHO) as “intratubular” or “diffuse” (Kennedy et al., 1998). Tubular structures that transitioned to a diffuse form due to invasion were designated as “intratubular/diffuse”. Tumor germ cells, which morphologically resemble the classical type, that lined the tubular structures were PAS-positive and evaluated as carcinoma in situ (CIS).

Results

In the study, seminomas were most commonly observed in the following breeds, respectively: Terrier (n:10), mixed breeds (n:3), German Shepherd (n:2), Boxer (n:2), Golden Retriever (n:2), Bulldog (n:1), Pekingese (n:1), Doberman (n:1), and Akita (n:1). Breed information was not available for seven dogs. Except for one case aged three

years, seminomas were observed between the ages of 7 and 21, with an average age of 11.6 years. In our study, only two of the testicles with seminoma were cryptorchids, and one of them was observed at the necropsy. Information on the breed, age, cryptorchidism, material data, and diagnoses was given in the table (Table 1).

Table 1. Breed, age, material data of the case.

Case Number	Dog data		Material Data		Tumor Type
	Breed	Age	Cryptorchidism	Operation/ Necropsy material	
1.	Bulldog	3	Cryptorchidic testicle	Necropsy	CS/SS
2.	(N)	13	Testicle (N)	Operation	SS
3.	Mix	11	Testicles	Operation	CS
4.	Terrier	12	Testicle (N)	Operation	SS
5.	Golden retriever	7	Right Cryptorchidic testicle	Operation	SS
6.	Pekingese	13	Testicle (N)	Operation	SS
7.	Doberman	11	Testicle (N)	Operation	SS
8.	Golden retriever	9	Testicles (N)	Operation	SS
9.	Terrier	15	Testicles (N)	Operation	SS
10.	(N)	9	Testicles (N)	Operation	SS
11.	Terrier	(N)	Left testicle	Operation	SS
12.	Akita	11	Left testicle	Operation	SS
13.	German shepherd	9	Testicles (N)	Operation	SS
14.	(N)	9	Testicle (N)	Operation	CS
15.	Terrier	21	Testicle (N)	Operation	SS
16.	(N)	10	Testicle (N)	Operation	SS
17.	(N)	12	Testicle (N)	Operation	SS
18.	Jack Russell terrier	8	Testicle (N)	Operation	CS/SS
19.	(N)	8	Scrotum	Operation	SS
20.	(N)	13	Testicles	Operation	SS
21.	Terrier	13	Testicles	Operation	SS
22.	Boxer	12	Testicles	Operation	CS
23.	Yorkshire terrier	14	Testicle (N)	Operation	SS
24.	Mix	(N)	Testicle (N)	Operation	CS
25.	Mix	13	Testicle (N)	Operation	SS
26.	Terrier	9	Left testicle	Operation	SS
27.	Terrier	13	Testicle (N)	Operation	SS
28.	Terrier	13	Testicle (N)	Operation	SS
29.	Boxer	10	Right testicle	Necropsy	CS
30.	German shepherd	13	Testicle (N)	Necropsy	SS

(N): No-information; Testicle **(N):** No-information about whether they are a right or left testicle.

After PAS staining, cases with germ cells showing red-stained cytoplasm, PAS-positive, were classified as Classical seminoma (n: 5) (Figure 1), while those without staining, PAS-negative, were categorized as Spermatocytic seminoma (n: 23) (Figure 2). In two cases of seminoma, despite a morphological appearance closer to the spermatocytic classification, a certain degree of PAS-positive staining was observed. Therefore, these were indicated as Spermatocytic/Classical seminoma (n: 2) (Figure 3).

Classical seminomas (CS): The animals diagnosed with classical seminoma were in the age range of 9-12 years, with the breed of one case unknown, while two were of the Boxer breed and two were of mixed breed dogs. The tumor germ cells had a single morphological type. Germ cells with

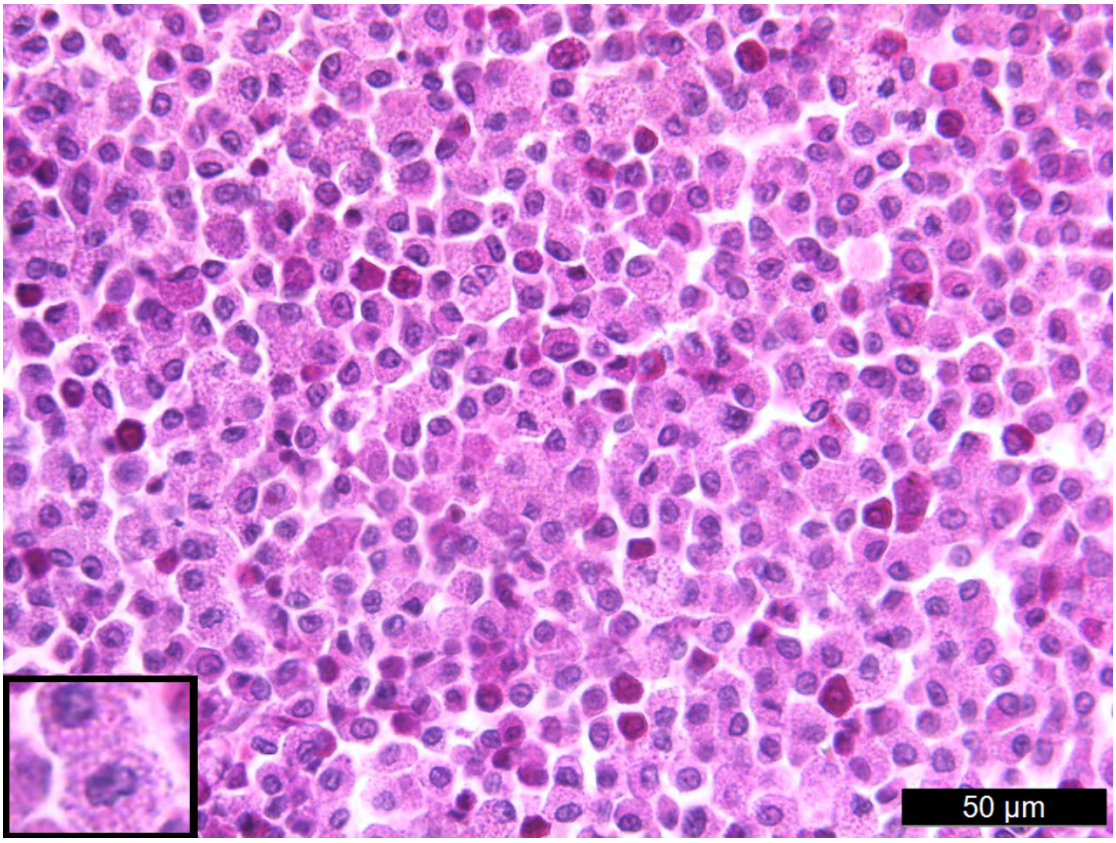

Figure 1. Classical seminoma (CS). Diffuse type seminoma composed of monomorphic neoplastic germ cells with PAS positive abundant cytoplasm (inset) and distinct border, PAS.

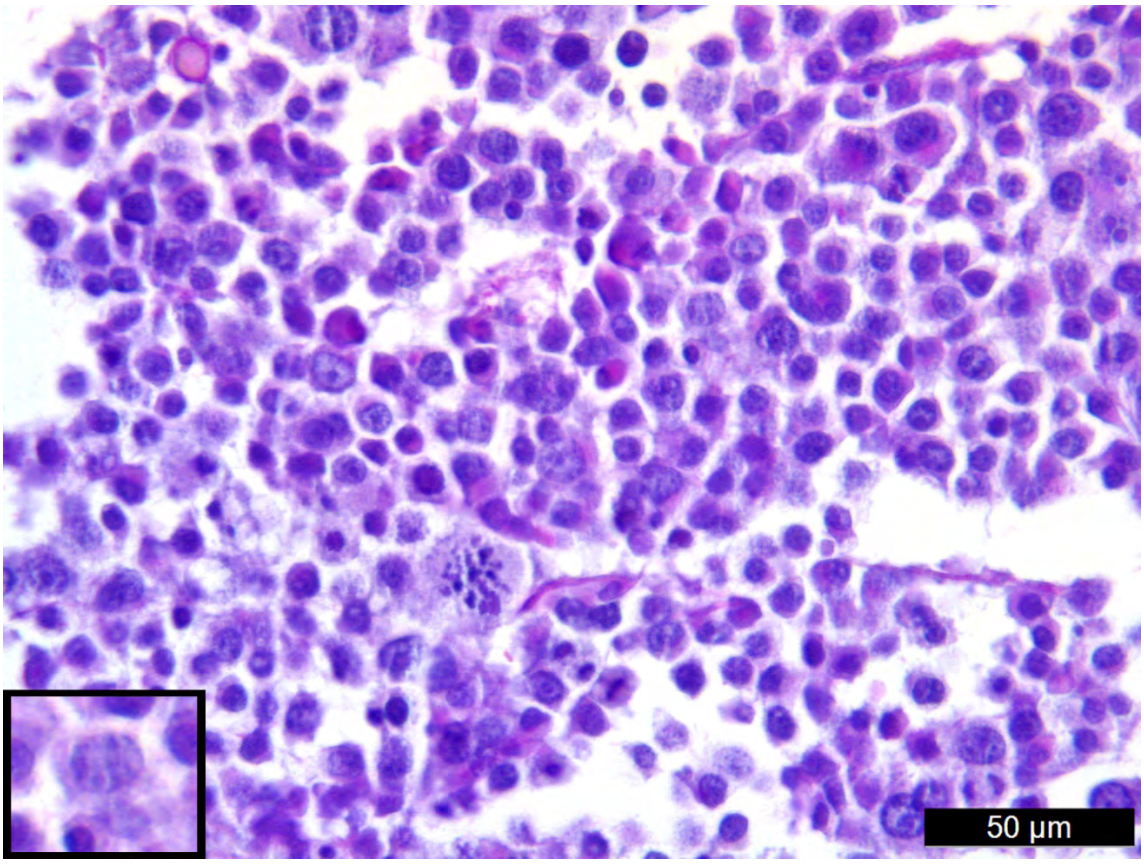

Figure 2. Spermatocytic seminoma (SS). Polymorph neoplastic cells with less abundant and PAS negative cytoplasms (inset), PAS.

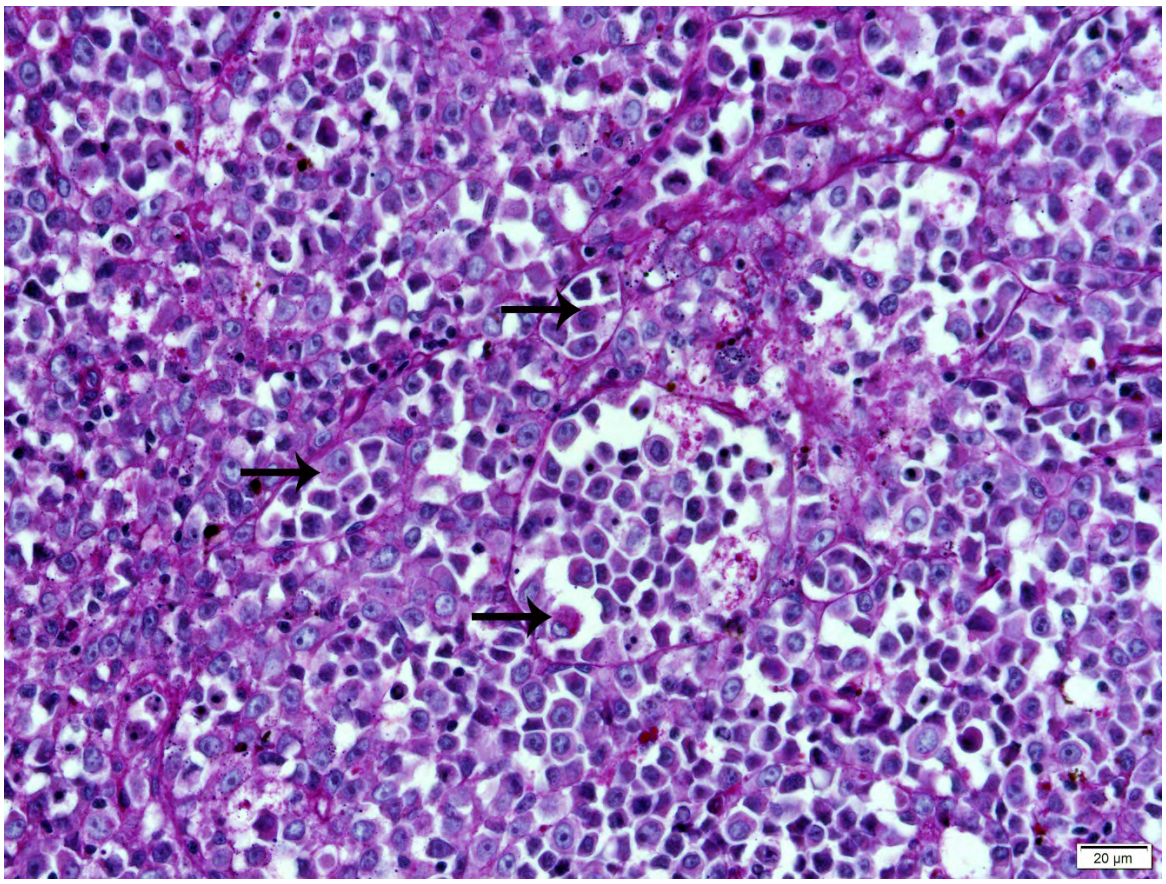

Figure 3. Spermatocytic/Classical Seminoma (SS/CS). The mixed type, consisting mainly of negative polymorphic neoplastic cells, and a few PAS-positive neoplastic cells with wide cytoplasm (arrows), PAS.

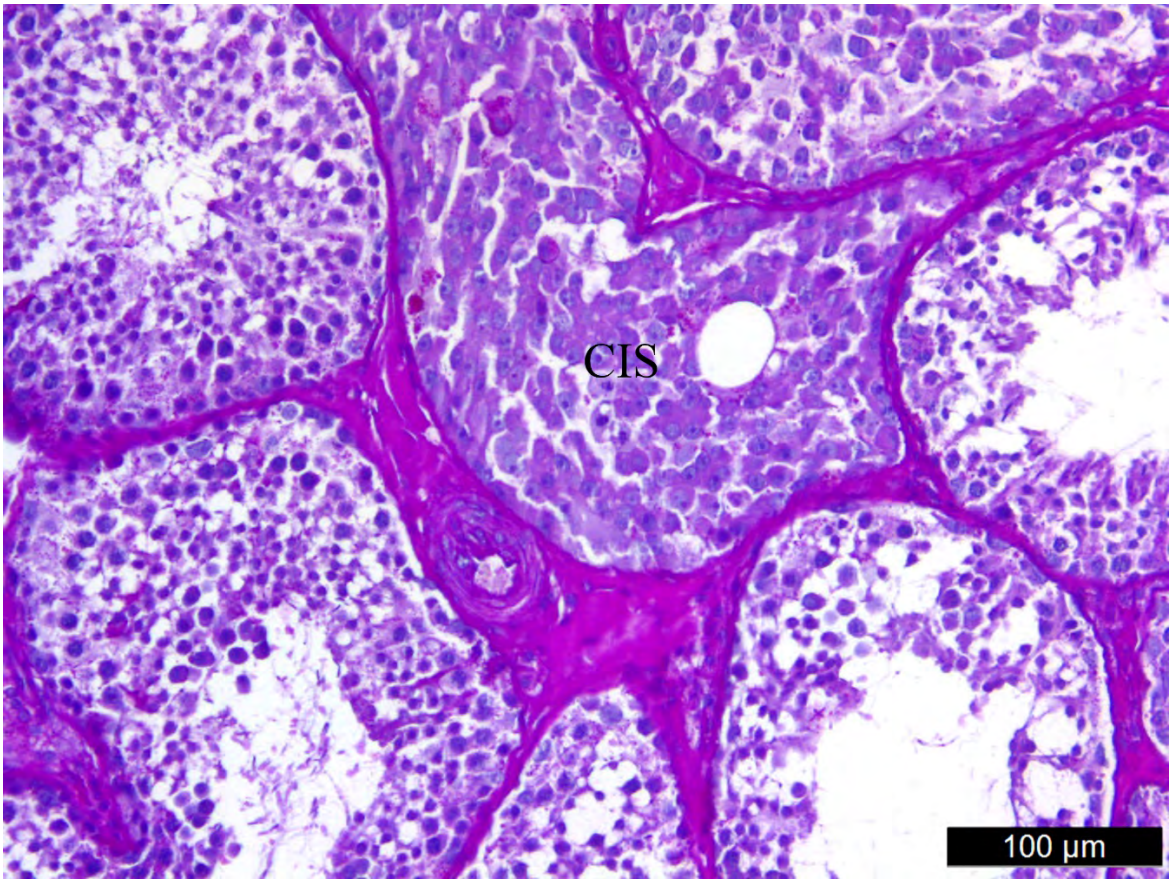

Figure 4. Testicular carcinoma in situ (CIS). Seminiferous tubule (CIS) filled by neoplastic spermatogonia, PAS.

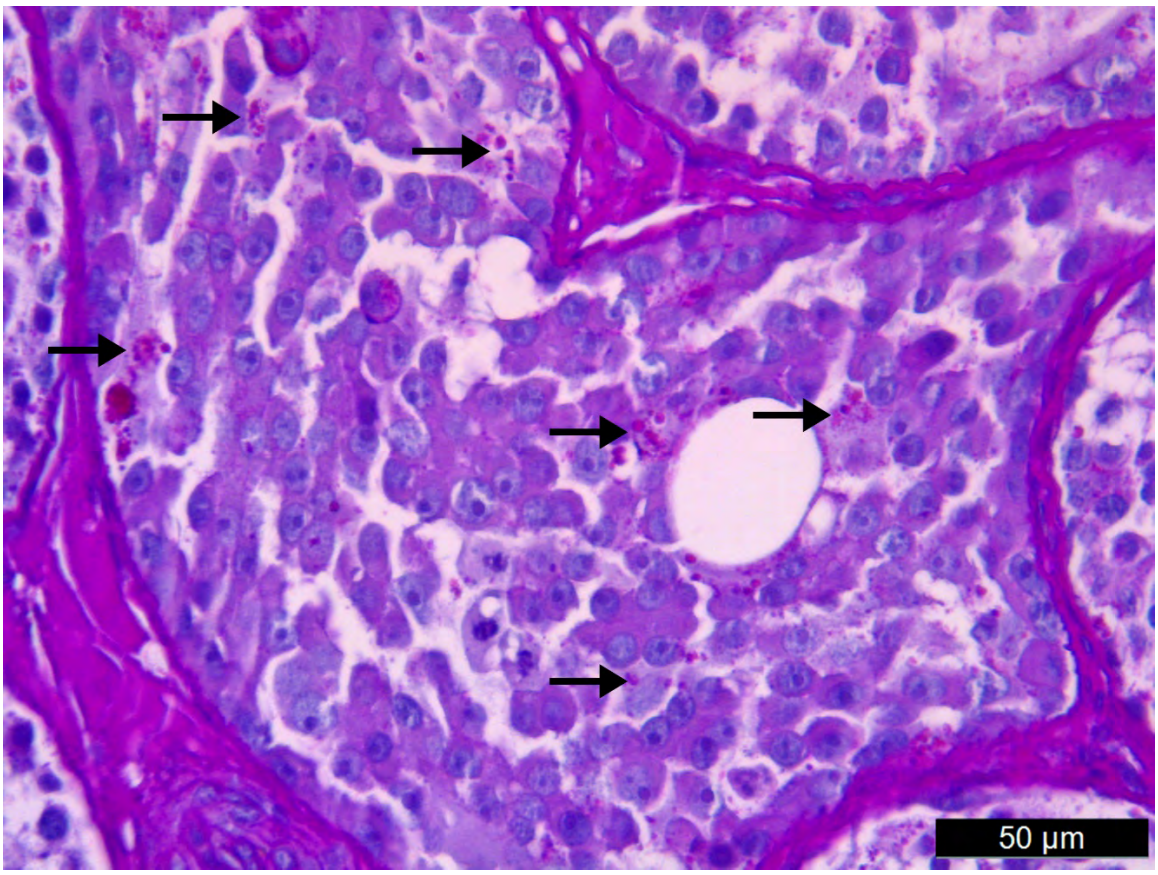

Figure 5. Testicular carcinoma in situ (CIS). Seminiferous tubule filled by neoplastic spermatogonia with PAS positive abundant cytoplasm (arrows) with well-defined boundaries, PAS.

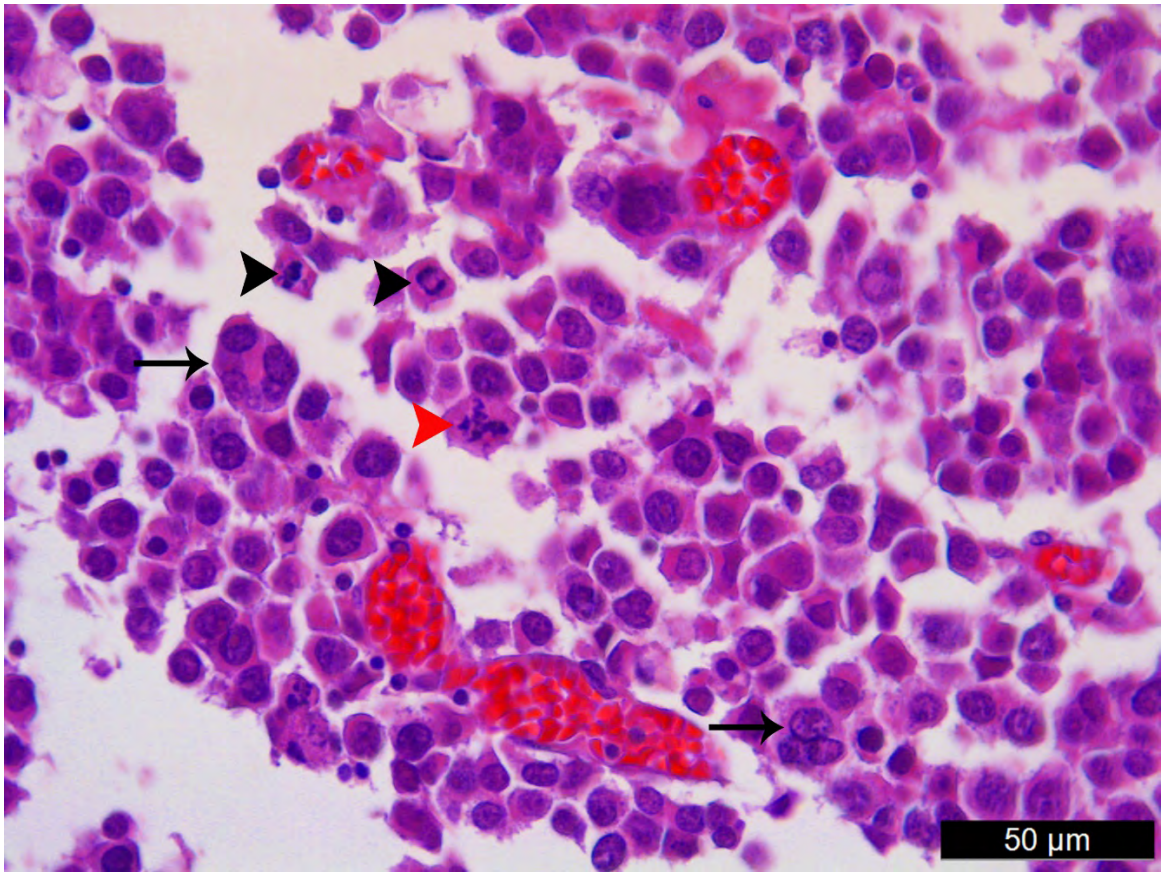

Figure 6. Spermatocytic seminoma. Giant cells (black arrows), mitosis (arrowheads) with spiremes type (red arrow), HE.

eosinophilic, abundant cytoplasm had well-defined boundaries. Giant cells were not observed in any case except for one. In all cases, the mitotic index was 1, and only one case showed a spiremes mitosis. While three cases had a diffuse pattern, an intratubular pattern was observed in two cases. Both cases with intratubular patterns were evaluated as Carcinoma in situ (CIS) (Figure 4 and 5).

Spermatocytic seminomas (SS): Animals classified as spermatocytic seminomas were in the age range of 7-21 years, with the majority observed in terrier-breed dogs (n=10). Among most spermatocytic seminoma cases, notable differences in cell size were observed among germ cells, leading to a characterization of polymorphism. Only two cases displayed a monomorphic appearance. Germ cells with amphophilic, less abundant cytoplasm had indistinct boundaries. Distinct cytoplasmic tumor cells were identified in one case. Giant cells were found in most cases (n=15). The mitotic index was mostly scored at 2 (n=12), and numerous spiremes mitotic figures (n=14) were encountered (Figure 6). Among the SS cases, the majority (n=12) displayed a typical intratubular pattern (Figure 7). In the remaining cases (n=10), although a diffuse appearance predominated, it was observed that the intratubular pattern turned into a more diffuse pattern due to tubular invasion, leading to an evaluation of the intratubular/diffuse pattern (Figure 8). One case exhibited only a diffuse pattern.

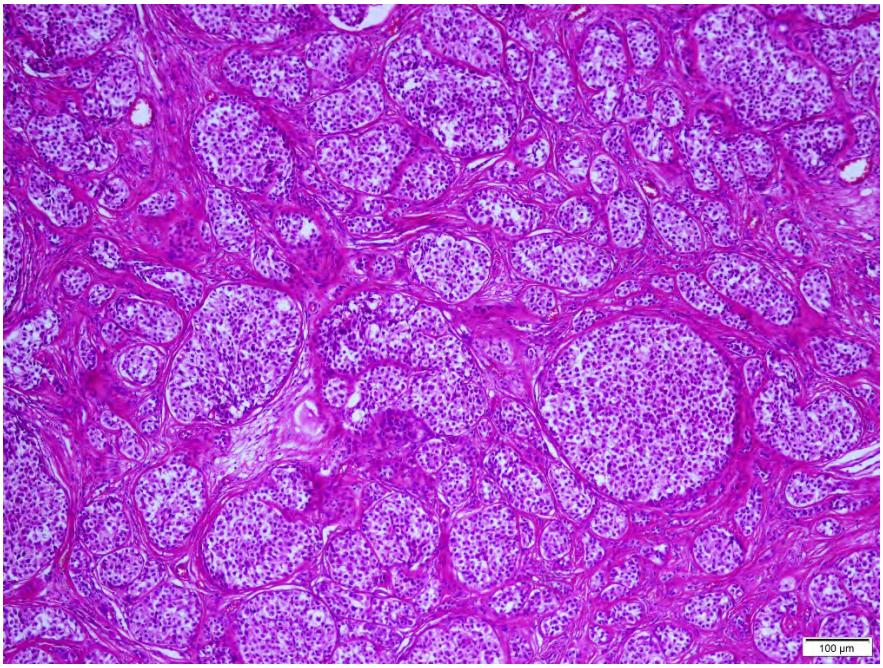

Figure 7. Spermatocytic seminoma. Intratubular type composed of neoplastic germ cells inside tubules, HE.

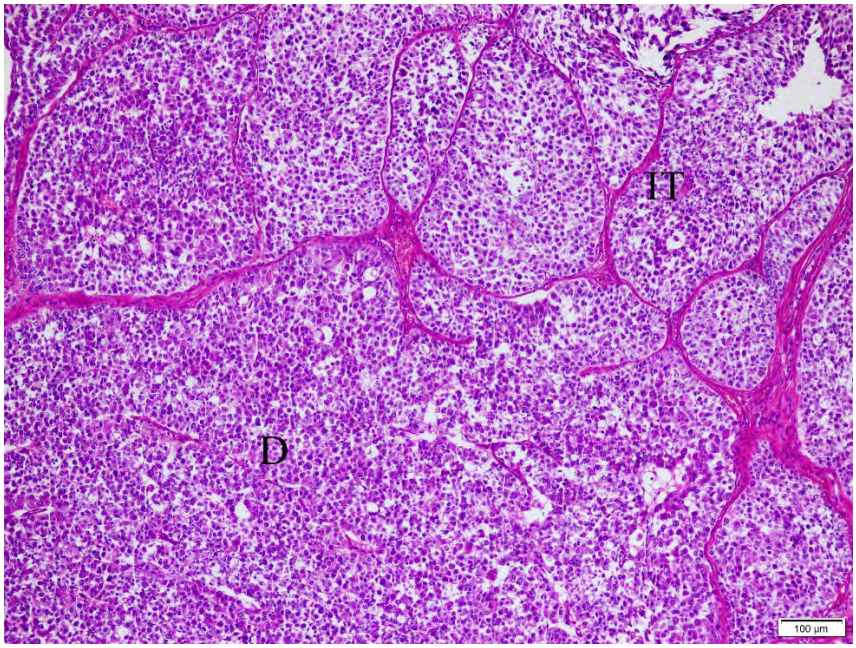

Figure 8. Spermatocytic seminoma. Intratubular/diffuse type composed of diffuse (D) and intratubular type (IT), HE." kullanılmalı.

Table 2. Tumor cell morphology, tumor structures and types of the cases.

Case Number	Tumor cell morphology									Tumor structure				Tumor Type
	Monomorphic	Polymorphic	Well defined border	Indistinct border	Abundant eosinophilic cytoplasm	Amphophilic less abundant cytoplasm	Present of giant cell	Mitotic index	Spireme mitosis	Intratubular	Diffuse	Intratubular /Diffuse	Present of CIS	
1.		+	+	+	+	+	+	3	+			+		SS/CS
2.		+		+		+		3	+		+			SS
3.	+		+		+			1				+		CS
4.		+		+		+		2	+	+				SS
5.		+		+		+	+	2	+			+		SS
6.		+		+		+	+	2	+			+		SS
7.		+		+		+	+	2	+			+		SS
8.		+		+		+	+	2		+				SS
9.		+		+		+	+	2	+	+				SS
10.	+			+		+		1		+				SS
11.		+		+		+		2	+	+				SS
12.		+		+		+		1		+				SS
13.		+			+	+	+	2	+	+				SS
14.	+		+		+			1			+		+	CS
15.		+		+		+	+	2		+				SS
16.		+		+		+		1				+		SS
17.		+		+		+	+	1				+		SS
18.		+		+		+	+	2	+		+			SS/CS
19.		+		+		+	+	2	+	+				SS
20.		+		+		+	+	1		+				SS
21.		+		+		+	+	2	+			+		SS
22.	+		+		+			1		+			+	CS
23.	+			+		+		1		+				SS
24.	+		+		+			1	+		+			CS
25.		+		+		+		1	+			+		SS
26.		+		+		+	+	3	+			+		SS
27.		+		+		+	+	1		+				SS
28.		+		+		+	+	2	+			+		SS
29.	+		+		+		+	1		+			+	CS
30.		+		+		+	+	2	+			+		SS

Spermatocytic/Classical seminomas (SS/CS): In two cases, prominent PAS-positive staining was observed, mostly PAS-negative. Notably, the cell morphology of the seminoma observed in the cryptorchid testis of a three-year-old animal exhibited features of both CS and SS classifications. The mitotic indices for both were 3, and they included giant cells and spiremes mitoses. One case exhibited a diffuse pattern, while the other displayed an intratubular/diffuse pattern.

Seminoma type and details regarding cell morphology of 30 cases of seminoma were given in the table (Table 2).

Discussion

The present study aimed to comprehensively classify and subtype 30 tumor cases using both Animal and Human World Health Organization International Histological Classifications (WHO) criteria. According to human criteria, seminomas are divided into two categories: classical seminoma (CS) and spermatocytic seminoma (SS) (Mostofy and Sesterhenn, 1998). Similar to a study conducted by Maiolino et al. (2004), these criteria were used to classify CS and SS subtypes in the present study. Additionally, we performed CS and SS subtyping using PAS staining like Grieco et al. (2007). In human medicine, CS was reported to be more common, while in veterinary medicine, SS was more frequently observed. In this present study, SS was the most prevalent seminoma subtype at 76.6%. In two cases, where negative staining was predominant but there was also a significant amount of positive staining, a third subtype was called as a mixed type (SS/CS). Particularly in one case, mixed cell morphology of both types was evident, suggesting that seminomas might originate concurrently from gonocytes and spermatocytes. This case in the present study, occurring in a young 3-year-old animal with a cryptorchid testis, raised the possibility of genetic predisposition and susceptibility to different, more aggressive tumor transformations under temperature influences. Exposure to this kind of effect such as post-natal radiation, chemicals have been reported to play an important role in the pathogenesis of CS (Grieco et al., 2007; Kristensen et al., 1996; Lamb et al., 1981). We recommend performing immunohistochemical PLAP staining in addition to PAS staining in cases where mixed cases (SS/CS) are suspected.

In human medicine, it has been reported that cases of CIS are only histopathologically observed in CS and not seen in cases of SS (Agnew and MacLachlan, 2016; Bush et al., 2011). In the present study, CIS formations were also observed in only CS cases. Similarly, studies have reported that CIS structures are the precursors of most germ tumors (Bush et al., 2011; Grieco et al., 2007; Skakkebaek et al., 1987). Even Grieco et al. (2007) mentioned that such CIS cases could be observed in other seminal tumors, suggesting a maldevelopment of testis and seminal epithelium. In this study, we thought that if there were more early diagnosis possibilities for SS/CS mixed cases, we could also encounter CIS cases in SS.

According to the animal classification, seminomas are subtyped based on their features into two distinct forms: intratubular and diffuse (Agnew and MacLachlan, 2016; Nielsen, 1998). Spermatocytic seminomas show intratubular growth pattern. Diffuse-type seminomas are more aggressive and are commonly encountered as the classic type in young men (Agnew and MacLachlan, 2016; Kennedy et al., 1998; Mostofy and Sesterhenn,1998). In our study, it was noteworthy that in 43.4% of SS cases, intratubular forms transformed to diffuse form by invasion and in fact, exhibited a predominantly diffuse structure. Parallel to the study conducted by Maiolino et al. (2004), the appearance in our study resembled the diffuse-dominant intratubular/diffuse pattern upon invasion and it is closer to the Classical form seen in humans. However, contrary to the findings of previous studies (Grieco et al., 2007; Grieco et al., 2008; Nascimento, 2020), only a small number of SS cases exhibited a solo diffuse pattern.

With a few exceptions, the cytomorphological appearances were consistent with findings from other studies and literature information (Agnew and MacLachlan, 2016; Akin et al., 2013; Bush et al., 2011; Grieco, 2007; Maoilino et al.,2004; Özsoy and Kutsal, 2007; Yumusak et al., 2014). However, unlike in human medicine, CS cases were not observed in younger animals (Agnew and MacLachlan, 2016). The polymorphic appearance, presence of giant cells, spiremes mitosis and high mitotic index were observed in SS forms, while the presence of a wide cytoplasm and monomorphic appearance with distinct borders were noted in CS cases, aligning with recent research and literature knowledge (Agnew and MacLachlan, 2016; Grieco, 2007; Maoilino, 2004). In the two cases with CS/ SS mixed subtype, it was noteworthy that the mitotic index score was consistently the highest (score:3). This aspect was striking and suggested that the mixed subtype might be even more aggressive.

The age and breed range also aligned with the findings from previous studies and literature information (Agnew and MacLachlan, 2016; Grieco, 2007; Maoilino, 2004). However, the observation of 4 cases of CS in our study, with 2 in mixed breed dogs and 2 in Boxer breed dogs, while not statistically significant, suggested the need for considering the Boxer breed in this aspect.

In conclusion, this study demonstrated that alongside CS and SS classification, the SS/CS mixed subtype can also be observed. It was emphasized that the SS/CS mixed subtype, which exhibited a small amount of PAS positivity, might encounter CIS structure in the SS type in its early stages. In addition to PAS staining for diagnosis, PLAP immunohistochemical staining is recommended. It should be noted that spermatocytic seminomas observed in an intratubular pattern generally tend to transform into a diffuse form.

Conflict of Interest

The authors stated that they did not have anyreal, potential or perceived conflict of interest.

Ethical Approval

This study is not subject to HADYEK permission in accordance with Article 8 (k) of the "Regulation on Working

Procedures and Principles of Animal Experiments Ethics Committees".

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Similarity Rate

We declare that the similarity rate of the article is 7% as stated in the report uploaded to the system.

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Author Contributions

Motivation / Concept: GYT
Design: GYT
Control/Supervision: AST, GYT
Data Collection and / or Processing: GYT, AST
Analysis and / or Interpretation: GYT, AST
Literature Review: GYT, AST
Writing the Article: GYT
Critical Review: AST

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