

# Trauma-induced testicular germ cell tumor in Priangan ram: a histopathological study

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**Abstract.** Testicular germ cell tumors (TGCT) are a rare group of neoplasms originating from the uncontrolled growth of germ cells within the testes. These tumors can significantly disrupt reproductive function by interfering with normal spermatogenesis, the process of sperm production. This case report details a unique instance of a TGCT in a young, 2-year-old Priangan ram, initially suspected based on clinical findings of testicular abnormality. Histopathological examination revealed the presence of a seminoma, a specific type of TGCT, encompassing the entire seminiferous tubule, the site of sperm development within the testes. This extensive involvement suggests a potential for substantial disruption of the ram's fertility. The observed seminoma displayed classic and spermatocyte subtype characteristics, adding complexity to the case. Trauma resulting from ram fighting is suspected as the underlying cause of this TGCT, highlighting a potential environmental factor in the development of these tumors. This report contributes valuable information to the limited body of knowledge surrounding TGCTs, emphasizing the importance of considering these neoplasms in the differential diagnosis of testicular abnormalities and recognizing their potential impact on male reproductive health.

## 1 Introduction

Testicular problems in livestock, particularly in species such as cattle, horses, rams, and pigs, are significant clinical concerns due to their potential impact on fertility and overall health. The testes are anatomically complex organs composed of primary cell types, including germ cells, Sertoli cells, Leydig cells, peritubular myoid cells, macrophages, and epithelial cells. Each cell type has distinct roles in testicular physiology, with germ cells essential for spermatogenesis. In contrast, Leydig and Sertoli cells provide structural support, hormonal regulation, and maintenance of the blood testis barrier [1,2].

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Accordingly, neoplasms in the testes can exhibit an expansive scope of histological variations depending on the sort of cells involved [1,2].

Research on testicular neoplasms in animals is notably limited; however, insights from well-documented human cases can provide valuable reference points for understanding the pathology, progression, and potential risk factors associated with these tumors in veterinary contexts. In human males, more than 90% of testicular neoplasms arise from germ cells, which line the lumen of the seminiferous tubules and develop into mature spermatozoa [3,4]. Despite their prevalence in testicular cancers, germ cell tumors (GCTs) remain relatively rare, comprising only 1-2% of all tumors in male populations [5]. Germ cell tumors are clinically significant due to their distinct histological subtypes—such as seminomas and non-seminomas—each with unique prognoses and therapeutic responses [3,5]. Furthermore, testicular trauma has been identified as a potential risk factor associated with the development of these tumors in some instances, suggesting that physical injury could play a role in pathogenesis [6].

In veterinary medicine, limited data exists concerning germ cell tumor incidence, histological characteristics, and risk factors across species. In particular, reports on germ cell tumors in rams, a species that may experience testicular trauma from specific behaviors like ram fighting, are scant. We report a case of a Priangan ram, a breed prone to such trauma, presenting with a testicular germ cell tumor. This case highlights the need for veterinarians to be aware of trauma as a risk factor for testicular neoplasia, especially in species with similar behavioral risks, and to recognize the histopathological characteristics critical for accurate diagnosis and management of this tumor type.

## 2 Materials and methods

A 2-year black-colored Priangan ram was brought to the West Java Animal Hospital, Cikole, Lembang, West Bandung Regency, West Java, Indonesia, on the 14th of March 2024 with reddish and enlargement on both testes. Based on the physical examination, the ram's body temperature was within the normal range of 38.9 degrees Celsius. The ram weighed 56 kg and had a body condition score (BCS) of 3 out of 5.

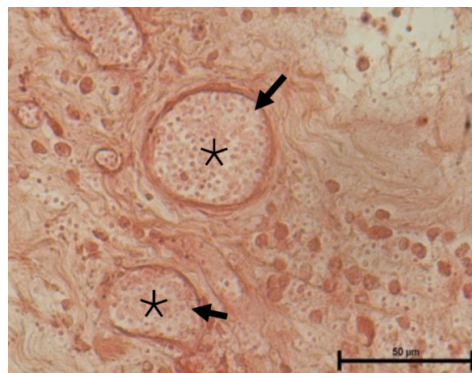


**Fig. 1.** (A) Macroscopic appearance of the Priangan ram's testis post-orchietomy; (B) Magnified macroscopic view of the Priangan ram's testis before orchietomy.

As a treatment for abnormal testicular conditions, the ram underwent an orchiectomy on the same day. Before the orchiectomy procedure, the ram was anesthetized using a combination of xylazine (Xyla®, Interchemie, The Netherlands) and ketamine (Ket-A 100®, Agrovet Market, Peru). The combination of xylazine (0.2 mg/kg body weight) and ketamine (10 mg/kg body weight) was administrated intramuscularly (IM), where xylazine was administrated before ketamine. Following anesthesia, the ram was positioned in the right recumbency and restrained using a rope. An incision in the distal third of the scrotum was made using a scalpel. The testis with tumors was separated from the fascia and cremaster muscle until the spermatic cord was visible. The spermatic cord is ligated using an absorbable suture (Catgut chromic suture, GEA Medical, China). When the orchiectomy procedure on both testes was completed, the scrotal skin was sutured in a simple interrupted pattern using a non-absorbable suture (Silk suture, GEA Medical, China). Tumor tissue samples are preserved using the Formalin-Fixed Paraffin-Embedded tissue method, in which the tissue is first fixed with formalin, then dehydrated through graded ethanol solutions, and cleansed with xylene. Subsequently, the tissue is incubated in liquid paraffin to be embedded into a block, thinly sectioned using a microtome. The resulting tissue sections are deparaffinized and rehydrated before being stained with hematoxylin and eosin. Histopathology examination was conducted at the Veterinary Center Subang, West Java, Indonesia.

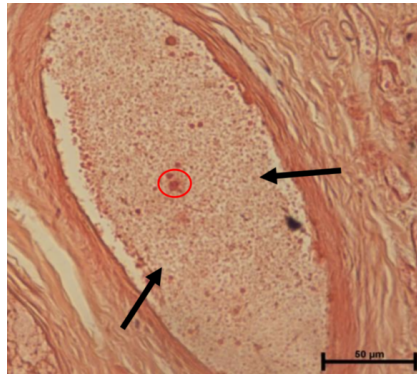
### 3 Results and discussion

Germ cell tumors (GCTs) are a group of neoplasms in the gonads, such as the testes and ovaries, originating from totipotent primitive germ cells that can be differentiated or undifferentiated [7,8]. Germ cell tumors arise from early embryonic stem cells or germ cells in various maturation phases [8]. Testicular germ cell tumors (TGCTs) have been described in multiple species, namely humans, dogs, horses, and goats, with varying histological findings in each case [9,10,11]. The current finding is the first reported testicular germ cell tumor in Priangan Ram; specifically, this case shows suspicion of trauma as the primary cause of the tumor. Germ cell tumors are heterogeneous in histology, location, and behavior [12,13].



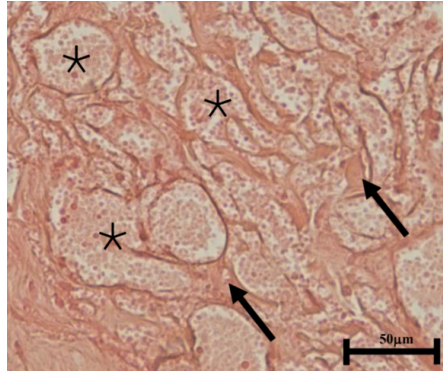
**Fig. 2.** Germ cell neoplasia (asterisk) shows seminiferous tubules filled with atypical germ cells located at the periphery of the seminiferous tubules (arrow). H&E staining. 40x magnification.

Histopathologically, in this case, neoplastic germ cells fill the whole seminiferous tubule, which forces transformations of the edge between the lumen of the seminiferous tubule and other tissue structures, therefore challenging to differentiate them (Figure 2). The presence of neoplastic germ cells in the seminiferous tubules, frequently called germ cell neoplasia in situ (GCNIS), is a typical precursor lesion in type 2 testicular germ cell tumors [8,14]. GCNIS is located in the basal lamina of the seminiferous tubules and looks comparable to primitive gonocytes. GCNIS will become an invasive germ cell tumor in nearly all similar cases [15]. What's more, the process of spermatogenesis, which is the activity of germ cells in various seminiferous tubules, has lessened or stopped. GCNIS is associated with impaired Sertoli cell (SCs) function, where naturally, Sertoli cells play a dominant role in spermatogenesis [14]. In this finding, germ cells can be identified through the enlarged white cytoplasm and discovered in the peripheral of the tubule, which follows the shape of the thickened germinal epithelial layer.



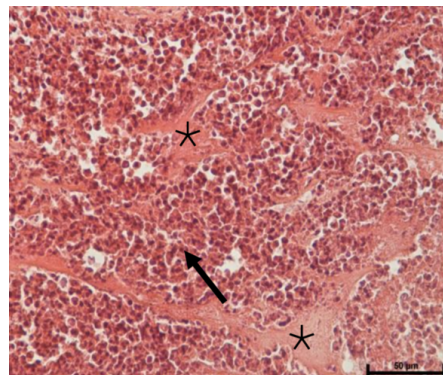
**Fig. 3.** The spread of seminoma cells throughout the tubules (arrow) is accompanied by giant cells in intratubular seminoma with multiple nuclei (circle). H&E staining. 40x magnification.

Seminoma development can be seen in the seminiferous tubules (tubular shape) and the interstitial tissue. Seminoma cells, which are malignant shifts of germ cells, have figure-like germ cells with modifications in dimensions to be larger, pale to clear cytoplasmic color due to glycogen accumulation, transformed cytoplasmic membranes, as well as enlarged cell nuclei, have one or more nucleoli, polygonal-shaped, and have flat edges [16, 17] (Figure 3). Regardless, seminoma cell development is not only in the peripheral region. The intratubular seminoma form will be discovered when seminoma fills the entire seminiferous tubule so that the spermatogenesis process stops and the border between the lumen and other tissues cannot be contrasted. The seminoma discovered has the characteristic of trabecular fibrovascular septa filled with lymphocytes, distinguishing between seminoma cells resembling nests or clusters (Figure 4). This follows the characteristics typical of classic seminoma tumors where a tumor arrangement comprises a band or nest formed by fibrovascular trabecular septa filled with inflammatory infiltrate in the form of lymphocytes that limit the seminoma cells [11, 18].



**Fig. 4.** Fibrovascular trabecular septa filled with lymphoid tissue (arrow) separate the seminoma cells (asterisk). H&E staining. 40x magnification.

Meanwhile, the differentiation of other tumor cells in the form of spermatocytic seminoma tumor cells was found in the interstitial tissue of the testis (Figure 5). Histopathological lesions are marked by the presence of polymorphic tumor cell proliferation, made up of tiny cells (similar to lymphocytes), eosinophilic and dense cytoplasm, and variable cell size with a spireme-type chromatin pattern (chromatin in the meiotic phase of spermatocytes) [16, 19]. As far as we know, this is the first case report revealing the combined presence of 2 major pathological categories of seminoma tumors: classical and spermatocyte. Classic seminoma is the most common testicular neoplasm that covers up to 50% of all germ cell tumors [20], while spermatocyte seminoma itself is unlikely and only covers 1% of testicular tumors [21]. This is unique because the histogenesis, clinical presentation, morphological features, and biological behavior of spermatocyte seminoma have no similarity to classical seminoma [22]. One tumor's cell morphology distinction suggests tumor heterogeneity [23]. Tumor heterogeneity is an attribute of malignant tumors that surface due to multiple divisions throughout growth [24].



**Fig. 5.** Spermatocytic seminoma characterized by the proliferation of polymorphic tumor cells (arrow) with formed trabeculae (asterisk).H&E staining. 60x magnification.

In several reports, the incidence of testicular tumors is often mentioned with trauma that occurs to the testes [25, 26, 27]. This happens since the testes must be outside the

abdomen to produce live and mature spermatozoa; consequently, they are prone to injury [6]. Concurrently, Priangan sheep are one of the sheep types often participating in the Priangan sheep agility art contest. In the agility art contest, sheep become susceptible to trauma due to fights between them. In these circumstances, severe injuries or trauma to the testes can cause testicular atrophy, which is a risk factor for testicular tumors [28].

Additionally, in traumatized tissue, the body will trigger several tissue repair mechanisms, one of which is the release of pro-inflammatory mediators [29]. The release of pro-inflammatory mediators, such as cytokines and chemokines, can promote tumors through several mechanisms, including DNA damage and angiogenesis [30]. Hence, trauma that may have occurred during the Priangan sheep fighting competition is suspected to be the cause of testicular tumors in this case.

## 4 Conclusions

This case appraises the occurrence of a testicular germ cell tumor in Priangan ram, confirmed through histopathological examination. With the finite number of reported cases, our case may imply that seminoma-type testicular germ cell tumors can present with tumor cell heterogeneity, as evidenced by classic and spermatocyte seminoma cells. Further, this finding underlines the potential for trauma to be a risk factor for testicular tumors.

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