CASE REPORT

Seminoma in a southern black rhinoceros (*Diceros bicornis minor*): diagnosis, surgical management and effect on fertility

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A testicular mass was identified by ultrasonography performed during a routine reproductive evaluation of an adult male southern black rhinoceros (*Diceros bicornis minor*). Histological examination of a testicular biopsy supported a presumptive diagnosis of testicular neoplasia. Hemi-castration was performed to excise the affected testis and a pathological diagnosis of a seminoma was made. Assessment of semen suggested reduced fertility as a consequence of the neoplastic process, but hemi-castration prevented further growth and metastasis of the tumour and ensured the animal's breeding potential. This is the second documented case of a seminoma in a rhinoceros species and the first case in a black rhinoceros.

Keywords black rhinoceros; fertility; hemi-castration; seminoma; testicular neoplasia

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Reproductive tract pathology, including cystic endometrial hyperplasia, cystic ovarian changes and uterine leiomyomas and adenomas, has been reported for captive female African (*Ceratotherium simum*, *Diceros bicornis*) and Asian (*Rhinoceros unicornis*) rhinoceroses[1,2] and these pathological changes contribute significantly to their suboptimal reproductive performance. In contrast, descriptions of pathological changes affecting the reproductive tract of captive male rhinoceroses are limited to age-related testicular fibrosis, without an apparent effect on fertility, and one report of a seminoma in an aged male white rhinoceros with intact fertilising capacity.[3–5] We report the diagnosis, surgical management and effect on fertility of a seminoma in a southern black rhinoceros (*D. bicornis minor*).

Case report

A captive-born, 12-year-old, male southern black rhinoceros weighing approximately 1200 kg was maintained at Taronga Western Plains Zoo, Dubbo, New South Wales, Australia as part of an ex situ assurance population for this endangered species. The rhinoceros was housed individually in a 0.25-ha yard within a purpose-built breeding facility. One other adult male, five adult females and five sub-adult black rhinoceroses were also housed within the breeding facility and adjacent public exhibit. The rhinoceros was fed a diet of meadow hay, lucerne hay and chaff, fresh produce and locally available browse species. It had sired one offspring and had shown no significant health problems prior to the diagnosis of the seminoma.

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Reproductive evaluation

Reproductive evaluation of the rhinoceros was performed under anaesthesia on four separate occasions: 12 months prior to tumour diagnosis, twice during the period of tumour characterisation and 10 months after surgery. Anaesthesia was induced on each occasion using a combination of 3.5 mg etorphine hydrochloride (Etorphine, Vericore Ltd, Dundee, Scotland, UK), 60 to 80 mg azaperone (Stresnil, Boehringer Ingelheim, North Ryde, NSW, Australia) and 5000 international units of hyaluronidase (Hyaluronidase, Kyron Laboratories, Benrose, South Africa) administered intramuscularly via projectile syringe (Dan-inject ApS, BK-7080 Børkop, Denmark). Physiological variables, including heart and respiratory rates, percent oxygen saturation and end-tidal CO_2, were monitored throughout the procedures. At the conclusion of each procedure anaesthesia was antagonised with 150 mg naltrexone (Naltrexone, Kyron Laboratories) administered intravenously.

Reproductive evaluation of the rhinoceros included transrectal and transcutaneous ultrasonographic examination of the accessory sex glands and abdominal testes, respectively, using a B-mode ultrasound scanning system equipped with a 2- to 4-MHz convex array transducer (Sonosite Vet 180PLUS, C60 probe Product Group International, Lyons, CO, USA) while the rhinoceros was in lateral recumbency. Additionally, semen (Table 1) was assessed following collection using a combination of electroejaculation with a specifically designed rectal probe that was positioned over the accessory sex glands via ultrasonography and manual massage of the penile and pelvic urethra.[4] Semen was collected into sterile, warmed collection vials, diluted 1:1 with a DMSO (Sigma-Aldrich, Sydney, NSW, Australia) egg yolk extender and total and progressive motility was assessed on the warmed stage of a phase-contrast microscope.[4] A haemocytometer was used to determine sperm concentration. Ultrasonographic examination of the left testis in November 2005 revealed a heterogeneous mass of generally reduced echogenicity with multiple hyperechoic foci that obliterated much of the caudal testicular parenchyma (Figure 1). A uniformly hypoechoic, smaller, spherical mass was evident cranial to the main mass. In order to further characterise the testicular mass, repeat anaesthesia was planned for a testicular biopsy.

Testicular biopsy

Four days after the initial examination, anaesthesia was induced as previously described, the rhinoceros was positioned in right lateral recumbency, intubated with a cuffed 26-mm endotracheal tube and anaesthesia maintained with 1% to 2% isoflurane (ISO Inhalation Anaesthetic, Veterinary Companies of Australia, Artarmon, NSW, Australia) in oxygen using a large-animal rebreathing circuit (Model VML, Matrix Medical Inc., Orchard Park, NY, USA) equipped with a

Table 1. **Ejaculate parameters prior to, at the time of diagnosis and subsequent to surgical removal of a unilateral seminoma from an adult male southern black rhinoceros**

Ejaculate parameter	Healthy (n = 1)	With seminoma (n = 2)	Hemi-castrated (n = 1)
Volume (mL)	47	9.5	16
Sperm concentration (10^6/mL)	70	225	6
Total motility (%)	90	57	10
Progressive motility	85	38	0
Intact sperm	75	30	5

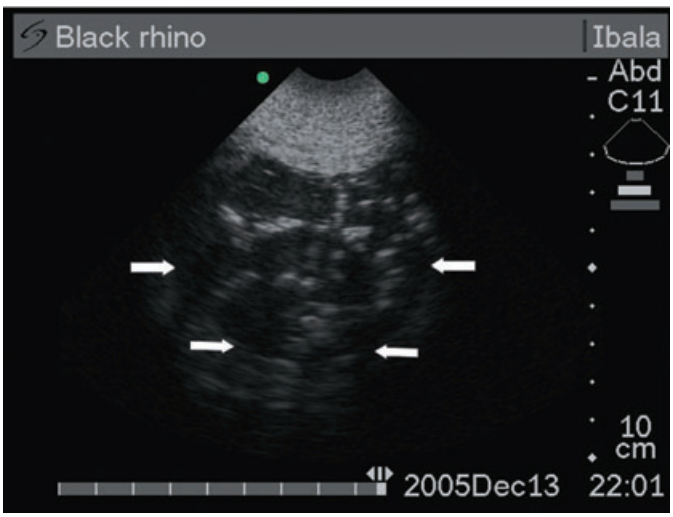

Figure 1. **Ultrasonographic appearance of the seminoma in the 12-year-old male southern black rhinoceros. Arrows delineate the margins of the tumour.**

30-L rebreathing bag. Following surgical preparation of the inguinal skin, ultrasound-guided biopsies of the mass in the left testis and the parenchyma of the right testis were obtained for further characterisation of the mass and to assess spermatogenesis in the unaffected testis. Biopsy material was collected using a 14-gauge needle trochar biopsy system (Pharmaseal Tru-Cut Biopsy Needles, Allegiance Healthcare Corporation, McGraw Park, IL, USA). Three biopsy samples from each testis were placed into Bouin's solution before routine processing for histopathological evaluation.

The histopathological results for the mass were consistent with a neoplastic process but, because the tissue architecture was poorly preserved, characterisation of the tumour type was not possible. Spermatogenesis within the seminiferous tubules was evident in the biopsy sample from the right testis. Given the rapid growth of the neoplastic mass and its apparent localisation in one testis, hemi-castration was selected as the most effective management option.

Hemi-castration

Four weeks after the histological diagnosis of testicular neoplasia, the rhinoceros was anaesthetised as previously described, intubated for

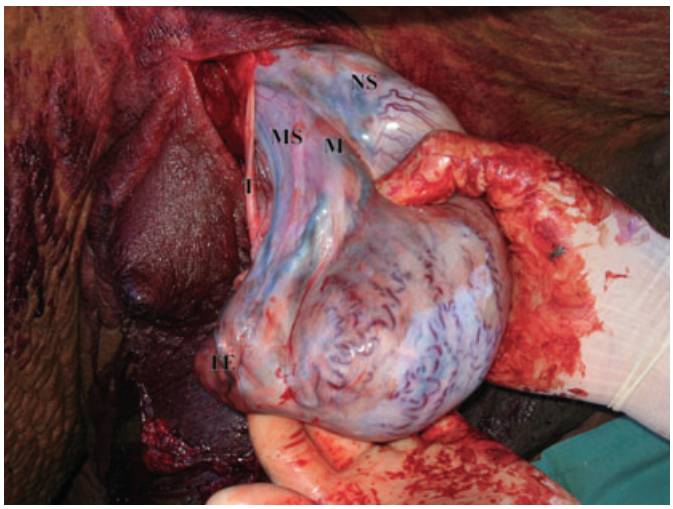

Figure 2. **Surgical approach to the hemi castration of a black rhinoceros with testicular neoplasia. Testis has been rotated 180° following eversion from the parietal vaginal tunic. M = mesorchium, MS = musculofibrous spermatic cord, NS = neurovascular spermatic cord, T = tunic, TE = tail of epididymis.**

gaseous maintenance of anaesthesia and positioned in right lateral recumbency. The location of the left testis was confirmed by ultrasonography. Following surgical preparation of the inguinal skin, a 10-cm incision was made through the epidermis and dermis and the soft tissue was bluntly dissected to the level of the parietal vaginal tunic. The tunic, which was tightly adhered to the surrounding tissue, was incised over the cranial pole of the testis and the left testis exteriorised (Figure 2). Digital pressure was used to penetrate the mesorchium to facilitate emasculation of the neurovascular component of the spermatic cord. The musculofibrous component was transected separately. Because of the risks of dust contamination and myiasis the skin incision was closed in multiple layers with polyglactin 910 (Vicryl® 1-0, Ethicon, San Angelo, TX, USA). Postoperatively the rhinoceros was administered trimethoprim (6 mg/kg) and sulfadimidine (30 mg/kg: Trimidine Powder, Parnell Laboratories Aust, Alexandria, NSW, Australia) orally twice daily and phenylbutazone (Butalone Granules, Apex Laboratories, Somersby, NSW, Australia) 2 mg/kg orally once daily for 5 days. Mild preputial swelling occurred 72 h after surgery, but resolved without complication over the next 7 days. At 10 days post surgery the skin wound partially dehisced and repeat anaesthesia was performed to remove the remaining sutures and flush the wound with a dilute chlorhexidine solution. Subsequently the wound was allowed to heal by second intention. No further problems occurred and wound healing was complete 4 weeks after surgery.

Gross pathology and histopathology

Approximately two-thirds of the cranial testicular parenchyma was obliterated by the neoplastic mass. The cut surface of the mass was pink, glistening, bulging slightly and had foci of mineralisation and necrosis (Figure 3a). Histologically, the testicular tissue had been replaced with broad sheets of densely packed epithelial cells with large, vesicular nuclei and scant quantities of eosinophilic to basophilic cytoplasm (Figure 3b). Moderate numbers of the epithelial cells were either multinucleated or had cleaved nuclei. Scattered through-

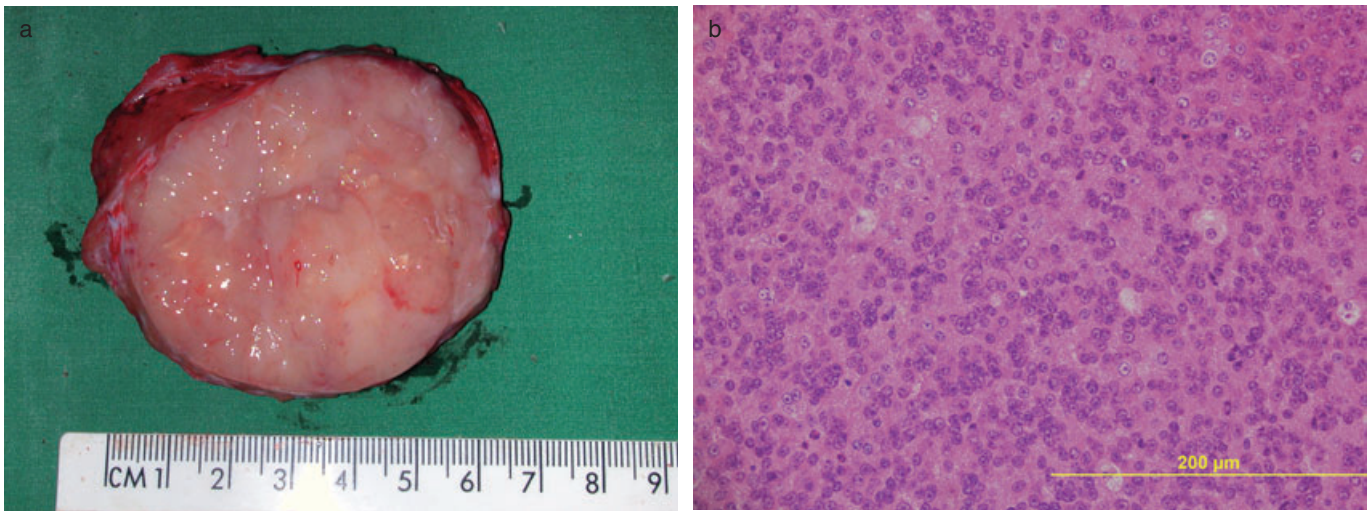

Figure 3. **Gross (a) and microscopic (b) appearance of a seminoma in a male black rhinoceros. (a) The cut surface of the seminoma glistens and bulges slightly with normal testicular parenchyma reduced to a thin rim around the outside of the tumour. (b) Densely packed epithelial cells with large, vesicular nuclei and scant quantities of eosinophilic to basophilic cytoplasm (H&E).**

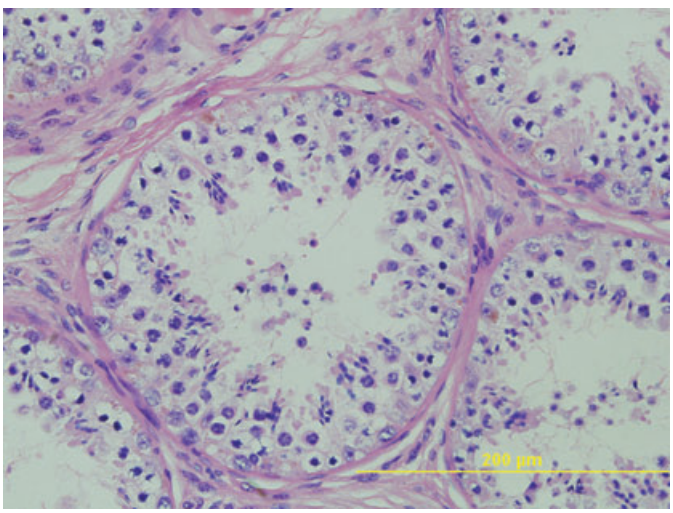

Figure 4. **Histological appearance of a seminiferous tubule from the neoplastic testis demonstrating spermatogenesis in the remaining normal testicular parenchyma (H&E).**

out the sections of the mass were small clusters of lymphocytes and occasional multinucleated giant cells containing abundant foamy eosinophilic cytoplasm. The sheets of epithelial cells were divided by multifocal connective tissue septae that occasionally contained large clusters of anaplastic epithelial cells unbounded by a basement membrane. Foci of coagulation necrosis, mineralisation and oedema were present within the interstitial connective tissue. Spermatogenesis was evident in seminiferous tubules adjacent to the tumour mass (Figure 4). Based on the histological findings, a seminoma was diagnosed.

Discussion

There is one other brief report of a seminoma in a rhinoceros, a captive southern white rhinoceros,[5] and the histological appearance of the seminoma in the present case was consistent with the previous findings. In both animals the seminoma appeared to grow rapidly, a feature common to seminomas in horses.[6] The Equidae and Rhinoceridae are related families within the taxonomic order Perissodactyla. In the present black rhinoceros, the seminoma was not evident on ultrasonographic examination performed 12 months earlier, but had obliterated much of the testicular parenchyma and exhibited necrosis and calcification at the time of diagnosis. In the previously reported case in a white rhinoceros, the seminoma grew substantially over the 11 weeks from the time of diagnosis. Those authors postulated that the seminoma may have developed subsequent to testicular trauma sustained during the establishment of a social hierarchy in a newly formed herd of white rhinoceroses. The black rhinoceros in this report had been maintained as a solitary animal for more than 18 months prior to the diagnosis of testicular neoplasia; however, localised swelling and a possible penetrating wound had been noted in the left inguinal region 10 months earlier. At the time the lesion was not investigated, but trauma as an inciting cause of the tumour is also possible in this case. Seminomas have been infrequently diagnosed in captive or free-ranging wildlife species and the diagnosis of a second case in a rhinoceros species is considered significant because it demonstrates that reproductive tract neoplasia also occurs in male rhinoceroses in captivity, albeit at a lower frequency than in females.

Ultrasonography is an effective imaging modality for examination of the gonads and accessory sex glands of rhinoceros species and can be performed in conscious, chute-trained animals or under anaesthesia for less tractable animals.[7] The ultrasonographic appearance of the present seminoma was similar to that described in a stallion[8] and a white rhinoceros,[5] with the exception of the multiple foci of mineralisation. Testicular biopsy was used as a further diagnostic tool for assessing fertility and for characterising the pathological processes affecting the testis; however, poor preservation of the tumour tissue architecture prevented a definitive diagnosis prior to surgical intervention.[9]

Surgical excision of the affected testis was selected as an effective option for managing the seminoma identified in this black rhinoceros. However, surgical intervention, even minor procedures, in rhinoceroses is problematic because of the potential problems associated with anaesthesia, the animal's size, the thick dermis and the inability to provide intensive postoperative care. In this case, intubation and gaseous maintenance of anaesthesia enabled a prolonged and controlled plane of restraint and analgesia during biopsy and hemi-castration. There is only one other account of surgical castration in a black rhinoceros, performed because of a possible genetic defect considered undesirable if perpetuated in a translocated population of free-ranging black rhinoceroses.[10] In that case, an open castration was performed, with each testis removed through an open incision following ligation of blood vessels, and the wounds were left open to heal by second intention, no postoperative complications being reported. In stallions, closed castrations are generally recommended when neoplastic testes are being removed.[6,11] In the present case, the parietal vaginal tunic was tightly adhered to the surrounding tissue, necessitating an open castration, and despite the creation of a large dead space, which was impractical to reduce following removal of the affected testis, the high risk of extensive postoperative dust contamination and myiasis necessitated primary closure of the skin wound. Problems encountered post surgery were mild preputial swelling, as is commonly seen in horses as a minor complication,[11] and breakdown of the surgical skin wound. These were considered to be relatively minor complications and the wound healed by second intention, with minimal scar formation, within 4 weeks of surgery.

Evaluation of semen collected via electroejaculation suggested declining fertility associated with the presence of the seminoma. Seminomas are not typically hormonally active and the effect on fertility was likely to be associated with replacement of the normal testicular parenchyma with neoplastic tissue. Histological examination of testicular tissue adjacent to the tumour mass revealed active spermatogenesis within the seminiferous tubules. Analysis of semen 12 months after hemi-castration revealed significantly lower values than those obtained prior to the diagnosis of the testicular tumour. However, the rhinoceros has subsequently bred with a female, resulting in a confirmed pregnancy. It is possible that one semen sample collected after the hemi-castration may not have been representative, because of the potential variables in the collection technique, or that the animal remains fertile despite significant lower semen parameters. Repeat collections would allow for better characterisation of this animal's semen quality.

In conclusion, a diagnosis of testicular neoplasia in a male black rhinoceros was made on the basis of ultrasonographic examination of the testis and ultrasound-guided testicular biopsy. Histological characterisation of the neoplasm as a seminoma was possible following surgical excision of the affected testis. Hemi-castration was an effective strategy for clinical management of this case and preserved the animal's reproductive potential.

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