



Immunohistochemical characterization of Sertoli cell tumour in an adult bull

SONU SHARMA^{1✉}, R P GUPTA¹, B L JANGIR¹, RAGINI HAZARI¹ and DEEPIKA LATHER¹

Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana 125 004 India

Received: 2 August 2020; Accepted: 6 September 2020

Keywords: Bull, Pancytokeratin, p53, Sertoli cell tumour, Vimentin

Testicular tumours are rare in most of the domestic animals except dog. These tumours include interstitial (Leydig) and Sertoli cell tumours, seminoma, teratoma, embryonal carcinoma, etc. Sertoli cell are tumours also known as sustentacular cell tumours and these originate from supporting cells within the seminiferous tubules. This tumour is common in dogs but has also reported in stallion, ram, cat and bull. These tumours usually occur unilateral in old animals but these have also been described in young calves (Meuten 2017). Immunohistochemistry plays an important role in identification and characterization of under diagnosed/ misdiagnosed tumours and tumours with specific genetic alterations associated with over expression or loss of expression of specific tumour markers (Kaspar and Crum 2015). Immunohistochemistry using a panel of antibodies have been used by earlier workers for confirmation of the histogenesis of primary testicular tumours in several animal species (Zanghi *et al.* 2004, Doxsee *et al.* 2006). In human and canine cancers a significant role of the p53 tumour suppressor gene in the tumorigenesis has been reported (Mayr *et al.* 1998) particularly in mammary tumours; however, the literature on bovine tumours is sparse. Keeping the above facts in mind, the present work was carried to study the immunohistochemical characterization of Sertoli cell tumour in bull using pancytokeratin, vimentin and p53 antibodies.

A six and a half years old bull was presented in the Department of Veterinary Clinical Complex, College of Veterinary Sciences of the University with history of gradual enlargement of both testes since last one and half years. The animal was getting weaker day by day. At the time of admission, the bull was anorectic and in a hide bound condition. Both the testes were enlarged (Fig. 1A), hard to touch on palpation and there was difficulty in getting up and down also. Animal was urinating normally. Both the testes were removed by open method of castration, and these were examined grossly. There was no septum between both testes. Although, the spermatic cords of both testicles were

separate but the testes were fused together without septum (Fig.1B) and was difficult to separate from the scrotum. These were hard in consistency and cut surface was uneven. The occurrence of this tumour in an adult animal in present study was in accordance with Meuten (2017) as he described that the testicular tumours in bulls are generally considered an age related problem. However, tumours in newborn and young calves also have been described (Palmer *et al.* 1980). Tumours of the testicles not only make the animal sterile but they are also life threatening. Similarly, in the present study, the bull was also losing body condition constantly. The bilateral tumour in the present case was contrary to earlier workers who reported that the Sertoli cell tumours in bovine are usually unilateral (Palmer *et al.* 1980, Foster and Ladds 2007).

The tumorous growths were collected in 10% buffered formalin and processed for paraffin embedding techniques by routine procedure (Luna 1968). For histopathology, the paraffin embedded tissues were cut into 4 µm thick sections using semi-automatic microtome and stained with haematoxylin and eosin (H&E). For immunohistochemistry, the sections were taken on 3-aminopropyl triethoxysilane coated slides and it was carried out as per procedure described earlier (Sharma *et al.* 2015). Primary monoclonal antibodies for pancytokeratin (anti-pancytokeratin, clone pck-26) and p53 (anti-p53, clone DO-7, Sigma Chemicals, USA) were used at the dilutions of 1:200. For pancytokeratin and p53 the secondary antibody and ExtrAvidin®-Peroxidase were used as per the manufacturer's instructions (Sigma). Amino-9-ethyl-carbazole (AEC; Sigma Chemicals, USA) was used as staining substrate. For vimentin primary antibody (anti-vimentin V9, Bio Genex), secondary conjugated antibodies (Bio Genex) and detection systems (Bio Genex) were used as per manufacturer's instructions. Mayer's haematoxylin (Sigma, MHS-16) was used as counter stain. Positive and negative controls were included in all reactions.

Histopathologically, the tumorous growth was diagnosed as Sertoli cell tumour. It was composed of neoplastic cells arranged in masses as broad sheets/ islands separated by connective tissue stroma (Fig.2A). These neoplastic cells showed indistinct cytoplasmic boundary, vacuolation in cytoplasm and large hyperchromatic nuclei

Present address: ¹Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125 004, Haryana, India. ✉Corresponding author e-mail: sonusharmavet@gmail.com

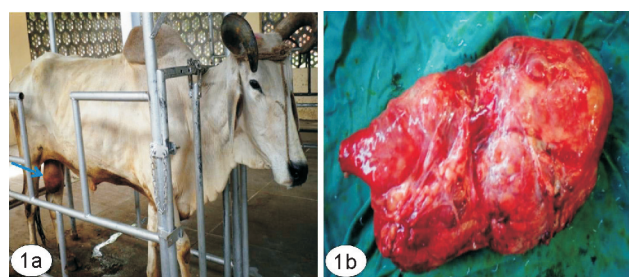


Fig. 1. (A) Sertoli cell tumour in a bull showing enlarged testes in the scrotum; (B) Testes fused together without septum.

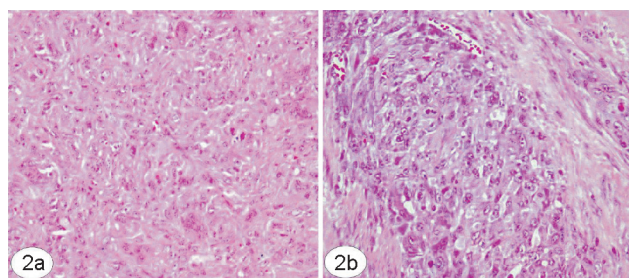


Fig. 2. (A) Sertoli cell tumour showing neoplastic cells arranged in masses as broad sheets/islands. (H&E×200); (B) Sertoli cell tumour showing pleomorphic hyperchromatic nuclei with prominent nucleoli and vacuolations in cytoplasm of neoplastic cells. (H&E×400)

with prominent nucleoli showing pleomorphism (Fig.2B). No mitotic figures were observed. Rounded /oval type germinal cells were not present. Absence of spermatogenic tissue in the present case was in agreement with the findings of earlier workers (Jensen *et al.* 2008) who reported a case of Sertoli cell tumour in a young bull along with areas of necrosis, haemorrhages and mineralization, though these secondary lesions were not noticed in the present study.

Sertoli cell tumour was characterized immunohistochemically using tumour markers for diagnosis and tumorigenesis. Pancytokeratin and vimentin immunostaining revealed that the neoplastic cells showed no reactivity for pancytokeratin and but strong intracytoplasmic staining for vimentin as reddish brown colour (Fig.3A). Vimentin is considered as important and most common intermediate filament in the cytoskeleton of Sertoli cells (Vogl *et al.* 1993). So, positive staining

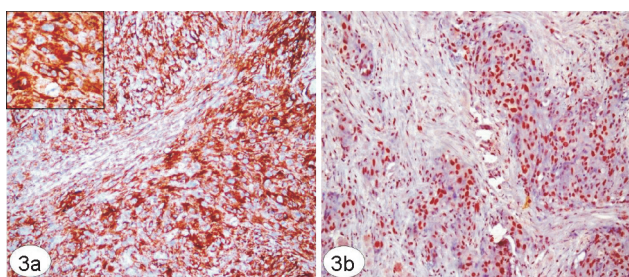


Fig. 3. (A) Sertoli cell tumour showing reddish brown colour intracytoplasmic immunostaining for vimentin (enlarged view in inset). (IHC×200); (B) Sertoli cell tumour showing reddish brown colour intranuclear immunostaining for p53 in neoplastic cells. (IHC×200)

for vimentin confirmed the histological diagnosis as Sertoli cell tumour. These observations are in accordance with earlier workers (Devkota *et al.* 2006) who reported the immunopositive reaction in normal Sertoli cells and tumour originated from these cells (Jensen *et al.* 2008) in bovine. A positive nuclear immunostaining to p53 as reddish brown colour was noticed. Most of the neoplastic cells exhibited moderate to strong staining intensity (Fig. 3B). No report could be traced on p53 immunohistochemical reaction in bovine but immunopositive reaction for p53 noticed in present study was consistent with findings of earlier workers in human beings (Takekawa *et al.* 1999, Guo *et al.* 2012).

In conclusion, it may be stated that these results provided evidence for the involvement of p53 mutants in development of Sertoli cell tumour. Application of pancytokeratin and vimentin antibodies provided important information for confirmatory diagnosis of Sertoli cell tumour.

SUMMARY

The present study reports a case of Sertoli cell tumour and its immunohistochemical characterization using pancytokeratin, vimentin and p53 in an adult bull. A six and a half years old bull was presented in the Department of Veterinary Clinical Complex, College of Veterinary Sciences of the University with the history of gradual enlargement of both testes since last one and a half years. Grossly, both testes appeared hard and firm. Microscopically, haematoxylin and eosin stained tissue sections revealed neoplastic Sertoli cells arranged in groups as islands/broad sheets separated by connective tissue stroma. Neoplastic cells showed indistinct cytoplasmic boundary, cytoplasmic vacuolation and enlarged pleomorphic hyperchromatic nuclei with prominent nucleoli. Neoplastic cells were positive for p53 and vimentin and negative for pancytokeratin. Moderate to strong p53 nuclear immunoreactivity was noticed in most of the neoplastic cells indicating role of p53 tumour suppressor gene in tumorigenesis. Vimentin immunopositivity was observed in cytoplasm of neoplastic cells but no immunoreactivity for pancytokeratin was noticed. The application of pancytokeratin and vimentin antibodies in present case provided important information for differential diagnosis between Sertoli cell tumour and seminoma.

ACKNOWLEDGEMENTS

The authors are thankful to Director, Teaching Veterinary Clinical Complex, LUVAS, Hisar for providing biopsy samples.

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