

Clinical and immunohistochemical evaluation of penile tumors in bulls

Research Article

Uğur Aydın^{1a}
Emin Karakurt^{2b}
Serpil Dağ^{2c}
Enver Beytut^{2d}
İsa Özaydın^{1e}
Hilmi Nuhoglu^{2f}
Uğur Yıldız^{1g}
Ayfer Yıldız^{2h}
Emre Kurtbaş²ⁱ

ABSTRACT

Fibropapilloma is the most common neoplastic formation associated with bovine papillomavirus type I. It occurs inside the penis of bulls and causes clinically adverse effects, such as phimosis, paraphimosis, urethral stricture, and urinary retention. This study aimed to conduct a clinical and immunohistochemical evaluation of penile tumors, which are associated with adverse outcomes with regard to breeding value and yield in bulls. A total of 40 bulls of different breeds aged between 10 months to 3 years were included in the study. Tumor tissue samples collected postoperatively were fixed in 10% buffered formaldehyde solution. The avidin–biotin–peroxidase method was used for immunohistochemical staining. Three separate areas were examined under 40X objective lens for each fibropapilloma tissue. The immunoreactivity was classified as none (-), mild (+), moderate (++), and severe (+++). Fibropapillomas ranged from 2 to 10 cm in diameter. Thirty-two papillomas were pedunculated and eight were sessile and attached to the body; 30 were solitary and 10 were multiple. There were 13 relapsed cases and 27 non-relapsed cases. Immunohistochemical examination revealed a statistically significant difference in the binary comparisons of proliferating cell nuclear antigen (PCNA) and hypoxanthine-guanine phosphoribosyltransferase (HPRT) based on positive cell scoring between the relapsed and non-relapsed cases. In conclusion, cancer markers such as PCNA and HPRT can be used to evaluate the prognosis and malignancy of penile tumors that cause significant economic losses.

Keywords: Fibropapilloma, cattle, hypoxanthine-guanine phosphoribosyltransferase, proliferating cell nuclear antigen, penile tumor.

INTRODUCTION

Fibropapilloma is the most common neoplastic formation associated with bovine papillomavirus type I. It occurs inside the penis of male cattle. Similar lesions can be observed on the vulvae and teats of cows in the same herd as an infected bull. Fibropapillomas, which generally occur at the tip of the penis, are more prevalent in young bulls aged <3 years than in older bulls due to the development of immune response by age. Penile tumors are associated with phimosis, paraphimosis, or penile prolapse. Furthermore, fibropapillomas with ulcerated surfaces can cause contamination through microorganisms and blood during cryopreservation of collected semen (Kamiloglu et al., 2004; Khodakaram et al., 2009; Kaya et al., 2010; Heppelman et al., 2019). Their attachment to the penile tissue can be pendulous or broad-based (Bulut and Ünsaldı., 2001; Heppelman et al., 2019). Although the fact that the cauliflower-like appearance of fibropapillomas during clinical observation may help with diagnosis, the definitive diagnosis is made following histopathological evaluation (Kaya et al., 2010).

¹Department of Surgery,
Faculty of Veterinary
Medicine, Kafkas University,
Kars, Türkiye, 36100
²Department of Pathology,
Faculty of Veterinary
Medicine, Kafkas University,
Kars, Türkiye, 36100

ORCID-

^a[0000-0001-5756-4841](https://orcid.org/0000-0001-5756-4841)

^b[0000-0003-2019-3690](https://orcid.org/0000-0003-2019-3690)

^c[0000-0001-7667-689X](https://orcid.org/0000-0001-7667-689X)

^d[0000-0003-3360-2940](https://orcid.org/0000-0003-3360-2940)

^e[0000-0003-4652-6377](https://orcid.org/0000-0003-4652-6377)

^f[0000-0003-2530-2542](https://orcid.org/0000-0003-2530-2542)

^g[0000-0002-4782-1012](https://orcid.org/0000-0002-4782-1012)

^h[0000-0002-6569-5435](https://orcid.org/0000-0002-6569-5435)

ⁱ[0000-0002-9752-194X](https://orcid.org/0000-0002-9752-194X)

Correspondence

Uğur AYDIN
uguraydin076@hotmail.com

Article info

Submission: 12-03-2022

Accepted: 24-07-2022

Online First: 06-08-2022

Publication: 31-08-2022

e-ISSN: 2548-1150

doi prefix: 10.31797/vetbio

• <http://dergipark.org.tr/vetbio>

This work is licensed under a
Creative Commons Attribution 4.0

International License



How to cite this article

Aydın, U., Karakurt, E., Dağ, S., Beytut, E., Özaydın, İ., Nuhoglu, H., Yıldız, U., Yıldız, A., Kurtbaş E. (2022). Clinical and immunohistochemical evaluation of penile tumors in bulls. *Journal of Advances in VetBio Science and Techniques*, 7(2), 210-219.
<https://doi.org/10.31797/vetbio.1086702>

Surgical removal may prove to be a better option in bulls that are intended for breeding on the grounds that spontaneous resolution of fibropapillomas may occur but at a slower pace. When untreated, pain and mechanical interference during copulation can adversely affect intromission and fertility (Bulut and Ünsaldı., 2001; Kamiloğlu et al., 2004; Heppelman et al., 2019). Treatment options such as excision, electrocauterization, and cryosurgery are used in the treatment of penile tumors (Kamiloğlu et al., 2004; Kaya et al., 2010; Heppelman et al., 2019).

The present study aimed to conduct a clinical and immunohistochemical evaluation of penile tumors, which are associated with significant economic losses in terms of breeding value and yield in bulls.

MATERIAL and METHOD

Animal material

The animal material of this study comprised 40 cattle presented to the animal hospital polyclinics of the Faculty of Veterinary Medicine, Kafkas University, with the complaint of a mass in their penis.

Clinical observations and treatment procedure

The bulls that were presented to the Animal Hospital of the Faculty of Veterinary Medicine, Kafkas University, underwent detailed clinical examinations. Bulls that were clinically diagnosed with tumors upon taking anamneses, as well as inspection and palpation examinations, underwent a surgical operation after the necessary preparations. Furthermore, the presence of tumor structures in different regions was investigated by inspection, rectal palpation, and radiographic examination prior to the surgical intervention. The inspection included the whole body along with the penis, radiographical examination included the lungs and chest cavity in particular, and palpation and ultrasound-guided rectal examination included the pelvic urethra, urinary bladder, kidneys,

abdominal cavity, spleen, and liver. After completion of all the clinical examinations, the animals were placed in the lateral position using Reuff's method following sedation by injecting 0.02 mg/kg IV xylazine HCl (Rompun® 2% 25 ml Bayer). Then, the penis was taken out of the preputium. The coarse dirt was removed by an assistant using antiseptic solutions. Then, 70% ethyl alcohol and 10% povidone-iodine (PVP-I) were used for the purposes of asepsis and antisepsis of the surgical site, which was thereafter isolated using surgical drapes. In addition to sedation, local infiltrative anesthesia of the penis was performed using lidocaine HCl (Vilcain® 50 mL, Vilsan). Following anesthesia, the mass in the penile tissue was removed using electrocautery. The mass was referred to the Department of Pathology for histopathological examination. Postoperatively, analgesics (0.5 mg/kg meloxicam-Anafleks® 0.5%, Hektaş) and antibiotics (procaine benzylpenicillin 200,000 IU, dihydrostreptomycin sulfate 200 mg, Reptopen® S, CEVA-DIF) were recommended for use for 3 days and 7 days, respectively, together with nitrofurazone ointment (Furacin® 0.2% Sanofi İlaç San. Tic. A.Ş.) for use around the penis and inside the preputium.

Tissue samples

The study material included samples from 40 cases of fibropapilloma, which were collected from the penile sites of bulls presented to the Department of Surgery and Pathology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey.

Histopathological examinations

Tumor tissue samples collected postoperatively were fixed in 10% buffered formaldehyde solution and embedded in paraffin blocks. Next, serial sections of 5-µm thickness were cut for hematoxylin and eosin (H&E) staining, where serial sections of 4-µm thickness were cut for immunohistochemical staining of poly-L-lysine-coated slides.

H&E staining was performed for the sections to investigate the histopathological changes, which were evaluated under a light microscope (Olympus Bx53). Images were taken using CellAP software (Olympus Soft Imaging Solutions, GmbH, 3.4).

Masson trichrome staining with aniline blue

The staining procedure was performed according to the manufacturer's instructions (Facepath, Barcode No: 8681065132824).

Immunohistochemical examination

The avidin–biotin–peroxidase method was preferred for immunohistochemical staining. The sections were deparaffinized for 10 min in three separate xylol series. The sections were rehydrated in graded alcohol series (100%, 96%, 90%, 80%, and 70%). The sections were soaked in distilled water for 3 min, followed by incubation for 20 min in 3% hydrogen peroxide solution to block endogenous peroxidase activity. The microwave method was used for the sections, which were soaked in phosphate-buffered saline (PBS) for 3 min to expose the antigenic receptors (Citrate buffer solution, pH 6, 800 watts, 10 min). After 20 min of cooling, non-immune serum (Thermo Scientific Histostain IHC Kit, HRP, broad spectrum, REF:TP-125-HL) was added to the sections for 10 min to prevent nonspecific staining. The primary antibodies, which were diluted at different ratios in PBS (anti-bovine papillomavirus [BPV], MyBioSource, MBS320197, dilution ratio 1/100; proliferating cell nuclear antigen [PCNA], Santa Cruz, SC-56, dilution ratio 1/100; and hypoxanthine-guanine phosphoribosyltransferase [HPRT], Bioss Antibodies, BS-9026R, dilution ratio 1/400), were then incubated in the fridge (4°C) overnight. Biotinylated secondary antibody (Thermo Scientific Histostain IHC Kit, HRP, broad spectrum, REF:TP-125-HL) was added to the sections for 10 min at room temperature, following which the sections were rinsed in PBS buffer three times for 3 min each. Next, all

the sections were incubated for 10 min using peroxidase-conjugated Strep Avidin (Thermo Scientific Histostain IHC Kit, HRP, broad spectrum, REF:TP-125-HL). The chromogen substrate 3,3'-diaminobenzidine-tetrahydrochloride solution (Thermo Scientific, REF:TA-125-HD) was incubated for 15 min by dripping into the sections. The sections, which were rinsed in distilled water for 5 min, were stained using Mayer's hematoxylin solution and covered with immune mount.

The preparations were observed under a light microscope (Olympus Bx53), and images were taken using CellAP software (Olympus Soft Imaging Solutions GmbH, 3.4). Detailed analysis of the images were performed using ImageJ (1.51j8).

Analysis of the immunohistochemical staining results of BPV, PCNA, and HPRT antibodies were performed upon investigation of immune positive reactions in the sites that most prominently reflected the staining character using a rating system based on the number of positive cells. To quantify the immune positive reactions in the tissues, the analyses were started based on the high-intensity reaction sites. Three separate areas were examined under 40X objective lens for each fibropapilloma tissue. Immunoreactivity was classified as none (-), mild (+), moderate (++), and severe (+++) (Beytut 2017).

Statistical analysis

Statistical Package for the Social Sciences (SPSS® 26.0, Chicago, IL, USA) software was used to analyze the results. The Mann–Whitney U Test was used in binary comparisons of PCNA and HPRT based on the positive cell scoring. The results were expressed as mean ± standard error (SE). A P value of <0.05 was considered statistically significant.

RESULTS

Clinical findings

The ages of the bulls ranged from 10 months to 2 years. Eighteen bulls were aged 2 years, 11 were aged 1 year, 7 were aged 1.5 years, 1 was aged 3 years, and 3 were aged 10 months. In addition, 19 bulls were of Brown Swiss breed and 21 were of the Simmental breed.

Pedunculated or sessile wart-like growth with a cauliflower-like appearance was observed during the macroscopic examination of the fibropapillomas. Some of the masses were highly hemorrhagic and necrotic. Most of the papillomas (32) were located at the tip of the penis (Figure 1A), and the others (8) were located in the penile body (Figure 1B).



Figure 1a. Image of tumoral mass in the glans penis, **b.** Image of tumoral mass in the penis body. Thirty were solitary and 10 were multiple.



Figure 2. Postoperative macroscopic view of the tumoral mass. The diameter of penile tumors ranged from 2 to 10 cm.

Upon inspection, palpation, ultrasonography, and radiographic examination for the presence of tumor formations, there were no tumor-like find.

Microscopic findings

Hyperkeratosis, acanthosis, and finger-like protrusions from the epidermis to the dermis and dense bundles of connective tissues between these structures that form vortex

structures were detected upon histopathological examination of the masses. Degeneration of keratinocytes, increase in the abundance of keratohyalin granules, and koilocytosis were observed. Other remarkable histopathological manifestations included inflammatory cell infiltration in the dermis layer, sporadic mitotic figures, hemorrhage and ulcers, and large necrotic areas (Figure 3-a-b).

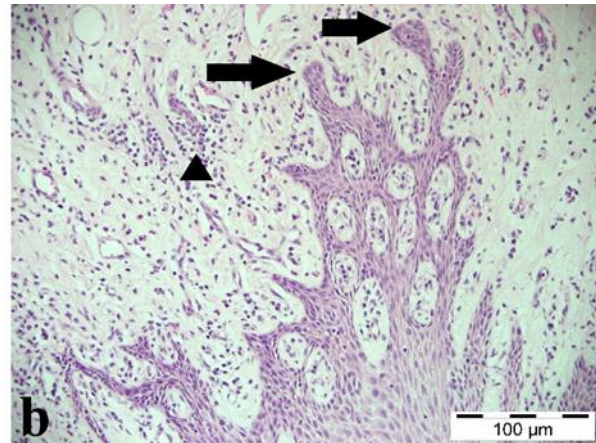
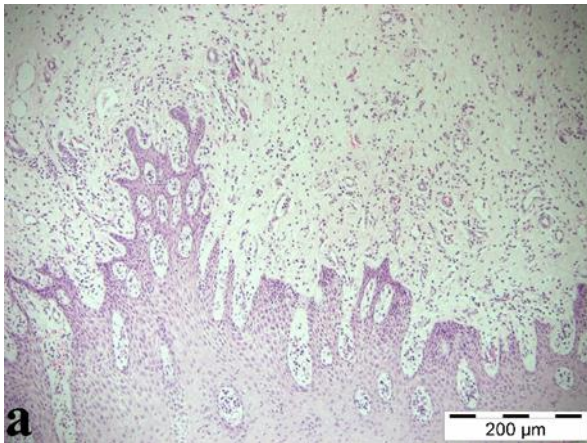


Figure 3a. Fibropapilloma, H&E, Bar= 200 μm, **b.** Higher magnification, rete pegs (arrows) and perivascular inflammatory infiltration (arrowhead), H&E, Bar= 100 μm

Masson trichrome staining with aniline blue

Masson trichrome staining with Aniline Blue indicated very intensive collagen accumulation in cases with fibropapilloma. There was

increased fibroblast activity in the rete peg areas and in the dermis immediately below the epidermis layer, and fibroblast activity was decreased toward the deeper sections of the masses (Figure 4 a-b).

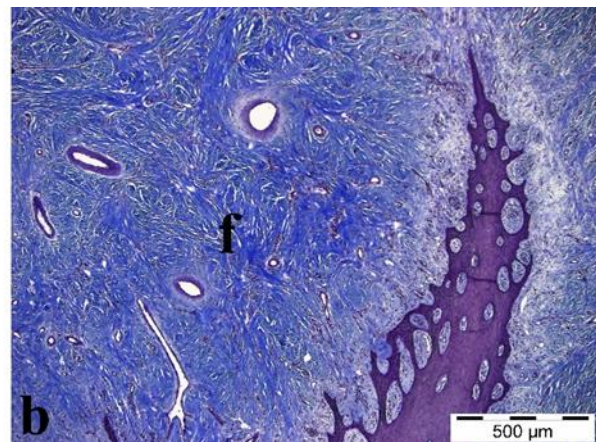
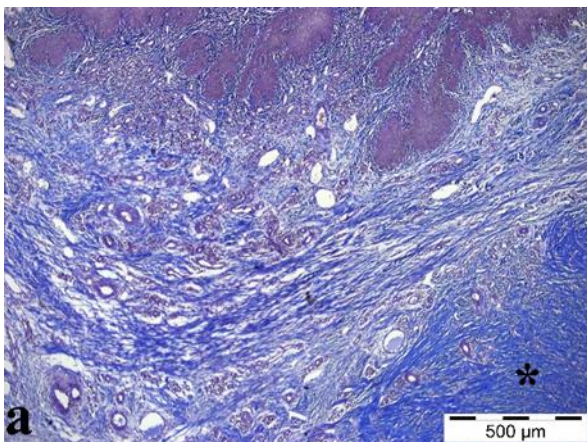


Figure 4. Masson Trichrome Aniline Blue Staining, **a-b.** Presence of connective tissue (star and f), Bar= 500 μm

Immunohistochemical findings

The number of positive cells in terms of PCNA and HPRT expressions of relapsed and non-relapsed cases after treatment are given in Table 1. It was determined that there was a

statistically significant difference in PCNA and HPRT expressions in the relapsed group compared to the non-relapsed group. Immunohistochemical examination revealed

BPV immune positive reactions in the nuclei of cells in the stratum granulosum layer of the epidermis (Figure 5-a-b). The PCNA immune positive reactions were more intense in epithelial hyperplasia and acantholytic areas. The PCNA cell expressions were especially yellow brownish in the nuclei of tumor cells in

the periphery of rete peg structures. The cellular proliferation of HPRT immune positive reactions were increased, characterized by epithelial hyperplasia, and more intense in the acantholytic sites. The HPRT cell expressions in a dark brown granular form were localized in the cytoplasm of fibrocytes and fibroblasts.

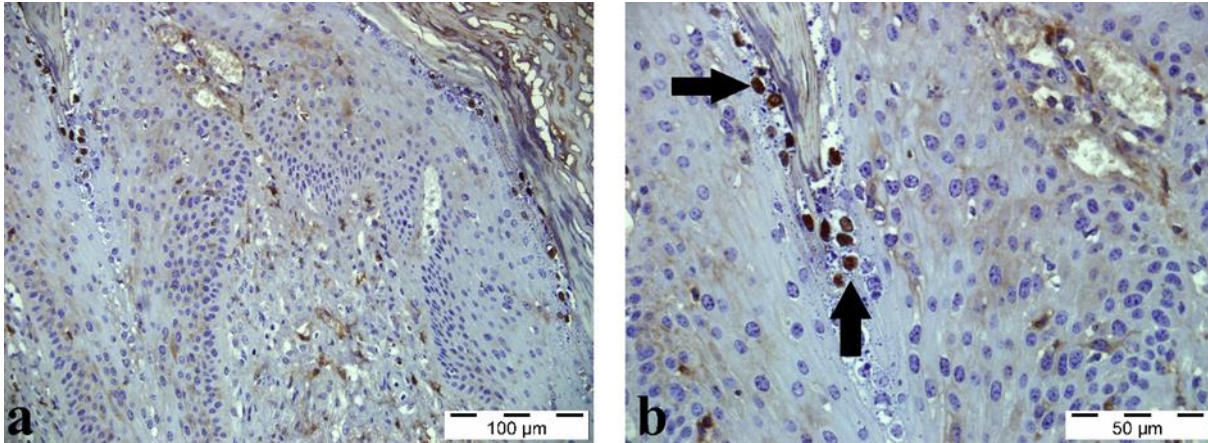


Figure 5 a. BPV immunopositive reactions in the nuclei of cells in the stratum granulosum layer, Bar= 100 μ m, b. Higher magnification, intranuclear BPV expressions (arrows), IHC, Bar= 50 μ m

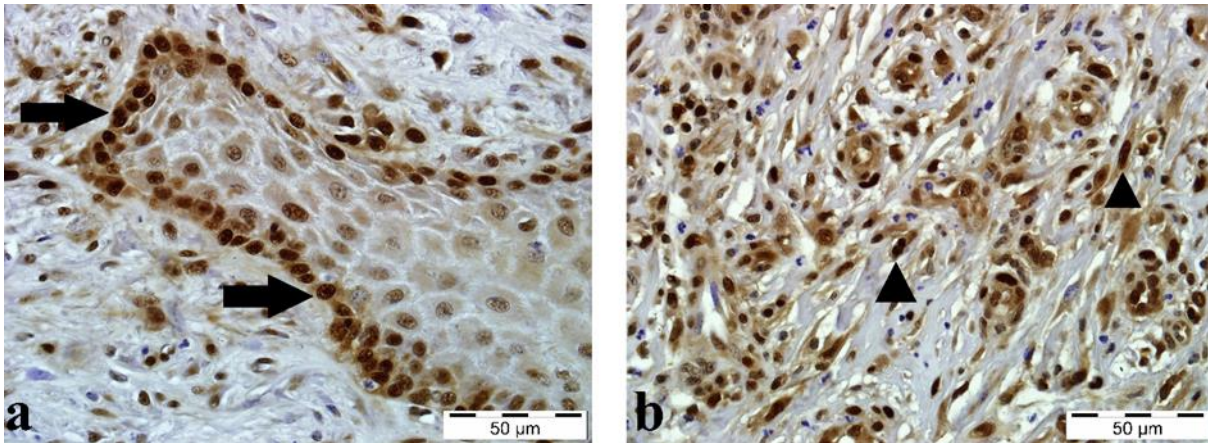


Figure 6 PCNA, IHC. a. Immune positive reactions in the nuclei of tumoral cells (arrows) in the periphery of the rete peg structures, Bar= 50 μ m, b. PCNA immunoreactivity in the nuclei and cytoplasm of fibrocyte and fibroblasts (arrowheads), Bar= 50 μ m

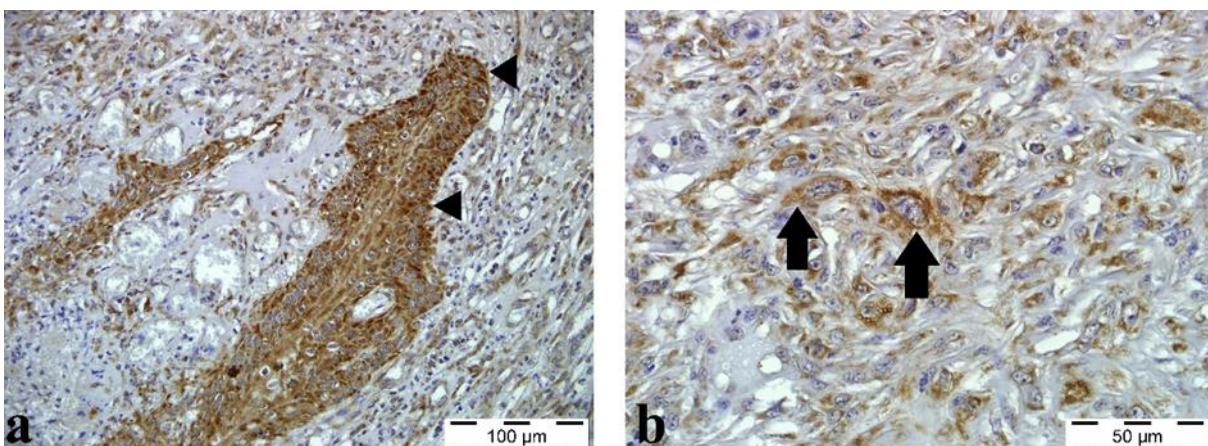


Figure 7 HPRT, IHC. a. Intense intracytoplasmic dark brown positive reactions in rete peg structures, Bar= 100 μ m, b. Granular HPRT expressions in the cytoplasm of connective tissue cells, Bar= 50 μ m

Postoperative period

All patient owners were contacted by phone for information. There were no relapses in 27 of the 40 cases, whereas there were 13 cases of relapse; 2 relapsed cases were re-operated, with no recurrence thereafter. In all the cases of relapse, the bulls were fattened for a period of 1–3 months and reserved for beef consumption. Eleven out of the 27 bulls without relapse after treatment were still used as breeders; there was no transmission to the offspring and their

mothers, and 16 bulls were reserved for beef consumption after an adequate fattening period of approximately 1 year. The three relapsed cases had urinary retention, whereas there were no urination-related problems in 37 cases.

Positive cell scoring

Immunohistochemical examination showed a statistically significant difference as a result of the binary comparisons of PCNA and HPRT by positive cell scoring between the relapsed cases and non-relapsed cases.

Table 1. Statistical analysis of PCNA and HPRT cell expression between relapsed and non-relapsed cases after treatment

Groups	PCNA	HPRT
Recurrence	2.85±0.38	2.77±0.44
No recurrence	1.41±0.50	1.19±0.40
P Value	<0.001	<0.001

DISCUSSION

Penis tumors in cattle are associated with significant economic losses if not treated in a timely manner. Therefore, it is important for the prognosis of the disease to choose both an effective treatment option and an advanced diagnosis method for penile tumors. A significant loss of productivity was prevented because of the treatment option and diagnostic method used in the present study and the results will contribute to clinical practice.

Fibropapillomas are benign tumors caused by papillomavirus; they originate from fibrocytes and fibroblasts and have a cauliflower-like appearance, as observed in the bulls, especially those aged 1–3 years, located around the penis, and preputium and in the vulva and teats of cows in the same herd as the infected bull. Fibropapillomas are usually localized at the junction of the glans and corpus penis and the craniodorsal part of the penis (Bulut and Ünsaldı, 2001; Biricik et al., 2002; Khodarakam and Kargar, 2009; Kaya et al., 2010; Heppelman et al., 2019). These tumors,

which are fed by vessels, may appear pedunculated or sessile (Bulut and Ünsaldı, 2001). In the present study, the fibropapillomas in the operated bulls occurred on the glans and corpus penis and had a cauliflower-like appearance. With regards to the 11 breeding bulls, which were also used for insemination, there was no post-treatment transmission in the mated cows and the offspring born from these animals. In 22 cases, there was a pedunculated attachment to the penile tissues, whereas in 18 cases, there was a sessile attachment. The majority of the 40 cases (36 cases) were aged 1–3 years. The fact that elder animals had a comparatively more advanced immune system may account for the fact that the vast majority of fibropapillomas occurred in younger bulls.

Although fibropapillomas can vary between a hazelnut and an apple in size, they may usually occur in a solitary or multiple structure (Bulut and Ünsaldı, 2001; Khodarakam and Kargar, 2009). In the present cases, fibropapillomas were closer in size to an apple in those with solitary structure, whereas those

with multiple structures reached the size of nuts or walnuts and in some cases were the size of an apple. The fact that the owners of the bulls failed to notice papillomas until hemorrhage and reluctance to copulate was observed might be the underlying reason for the large-sized fibropapillomas in the present cases. In addition, the owners of the bulls with papillomas with solitary structure, especially those on the glans, may not notice the condition until the penis hangs out due to the effect of gravity. This may result in excessive growth of papillomas.

Fibropapillomas on the glans caused urethral stricture, urinary retention, and even complete closure of the orificium urethra externa, and rarely led to ischemic necrosis upon combined pressure on the penis of preputium hair and tumor (Bulut and Ünsaldı, 2001; Kamiloğlu et al., 2004). Urinary retention was observed in only 3 of the 40 cases in the present study, whereas there was no stricture, closure, and resultant urinary retention in the other 37 cases.

Usually, electrocautery is performed for the treatment of penile tumors in bulls because this method is associated with a decreased incidence of hemorrhage and relapse. The tumor can also be removed using traditional surgical procedures. Nevertheless, the risk of hemorrhage and transmission is slightly higher in the traditional surgical methods (Bulut and Ünsaldı, 2001; Kamiloğlu et al., 2004; Kaya et al., 2010; Heppelman et al., 2019). In all the present cases, the tumor was removed using electrocautery. The reason for the small number of recurrent cases during the postoperative period can be considered the complete removal of the tumor using electrocautery.

Although penile tumors can be clinically diagnosed, the definitive diagnosis requires histopathological and immunohistochemical examination (Kaya et al., 2010). The macroscopic and microscopic characteristics of the fibropapilloma cases in the present study

were consistent with the data reported in the literature (Kelman, 1997; Jelinek and Tachezy, 2005; Hatipoğlu et al., 2009). Similar to that reported in previous studies (Biricik et al., 2002; Hatipoğlu et al., 2009), intranuclear BPV immune positive reactions were observed in cells in the stratum granulosum layer.

Proliferating cell nuclear antigen (PCNA) plays an important role in nucleic acid metabolism as a component of the replication and repair mechanism (Kelman, 1997). It has many functions, such as DNA repair, translation synthesis of DNA, DNA methylation, chromatin remodeling, and cell cycle regulation (Maga and Hübscher, 2003). Furthermore, it is used to investigate tumor cell proliferation and tumor malignancy (Hatipoğlu et al., 2009; Özsoy et al., 2011). Özsoy et al. (2011), investigated PCNA immunoreactivity in 2011 to determine cell proliferation in cases of bovine cutaneous papillomatosis. They found that the PCNA expression was more intense in epithelial hyperplasia and acantholytic areas. Similarly, in 2005, Jelínek and Tachezy (2005) reported that there was intensive PCNA immunoactivity in the basal layer of the epidermis and dermis in cases of bovine cutaneous papillomatosis. In 2007, Maeda et al. (2007) investigated PCNA expression to determine cell proliferation in cases of breast papillomatosis caused by papilloma virus type-6 and found that the reaction was more intense in the spinous layer of the epidermis and in the basal layer. Consistent with the results reported in the literature, there was intensive intranuclear PCNA expressions in the periphery of rete peg structures in the present study. This increase in PCNA expression indicates that fibropapillomas have a very high proliferative index. When the postoperative long-term outcomes in the present cases were evaluated, it was found that all relapsed cases were directly correlated with the increases in PCNA expression. There was a statistically significant difference upon binary

comparisons of PCNA cases with and without relapse by positive cell scoring.

Hypoxanthine-guanine phosphoribosyltransferase (HPRT) is a common marker used to determine the frequency of mutations in the development of cancer and potential carcinogens (Townsend et al., 2017). HPRT is a recovery pathway enzyme that is involved in the production of both guanine and inosine bases. The enzyme acts by transferring phosphoribose from phosphoribosyl diphosphate to hypoxanthine or guanine bases, forming inosine monophosphate and guanosine monophosphate, respectively (Townsend et al., 2017). Due to the constant need for guanosine-5'-triphosphate as both a nucleotide for DNA synthesis and an energy molecule, HPRT is reliably produced as a cleaning gene across the cell and is found at low levels in all somatic tissues (Townsend et al., 2019). HPRT has shown potential as a surface antigen in several malignancies, including lung cancer, colorectal cancer, and Burkitt's lymphoma, and is used as an important parameter in determining malignant structures along with increased metastasis and tumor proliferation. Nevertheless, there is no detailed study that fully explains the effect of HPRT on tumor proliferation and migration (Townsend et al., 2021; Wang et al., 2021). Townsend et al. (2018) investigated enzymes in a colorectal cancer cell line and reported that HPRT had excessive expression in cancer tissues compared with that in normal tissues because of its role in proliferation and cell cycle regulation. Townsend et al. (2019) investigated HPRT expression in benign and malignant cancer cases using immunohistochemical and PCR methods and reported that HPRT immunoactivity was more intense in malignant cancer cases than in benign cancer cases. Wang et al. (2021) found that HPRT supported proliferation and metastasis in head and neck squamous cell carcinoma by directly interacting with signal transducer and activator of transcription 3 and that suppression of HPRT

expression stopped cancer development. In addition, it was suggested that HPRT expression indicated poor prognosis. Similarly, Sedano et al. (2020) investigated the role of HPRT1 in breast cancer and found that HPRT1 expression was higher in tumor tissues than in normal tissues. As a result of the above research, it was concluded that there was an inverse correlation between HPRT expression and survival. Similar to the studies by Townsend et al. (2019), Sedano et al. (2020), and Wang et al. (2021) the present study investigated the immunoactivity of HPRT in cases of fibropapilloma, a benign tumor, and found that the reaction was more intense in sites with increased proliferation, characterized by epithelial hyperplasia, and in acantholytic sites. A review of the postoperative long-term outcomes of the cases suggested that there was a direct correlation between the increased HPRT and PCNA expressions and the relapsed cases. Furthermore, there was a statistically significant difference upon binary comparisons of PCNA cases with and without relapse by positive cell scoring.

CONCLUSION

In conclusion, it was shown that cancer markers, which are of great importance terms of the prognosis of penile tumors, which are among the most important causes that have a negatively impact on the breeding value and meat yield of bulls, are useful parameters in evaluating the malignancy of this disease. Moreover, the clinical results of the study will significantly contribute to clinical practice in terms of the prognosis of penile tumors and thus help with preventing the loss of productivity to a large extent.

ACKNOWLEDGMENT

The authors thank Assoc. Prof. Mushap KURU for his help to statistical analyses.

Ethical approval: The study was conducted following the approval of the Kafkas University Animal Experiments Local Ethics Committee (approval no: KAÜ-HADYEK-2020-172).

Conflict of interest: There is no conflict of interest between the authors.

REFERENCES

- Beytut, E. (2017).** Pathological and Immunohistochemical evaluation of skin and teat papillomas in cattle. *Turkish Journal Veterinary Animal Science*, 41 (2), 204-12.
- Biricik, H.S., Çimtay, İ., Keskin, O., & Baba, Z.F. (2002).** Effect of autogenous vaccine and autohaemotherapy combination in the treatment of penile fibropapilloma in a bull. *Veteriner Hekimleri Mikrobiyoloji Dergisi*, 2(2), 13-6.
- Bulut S., & Ünsaldı, S. (2001).** Operative treatment of penile fibropapillomas observed in the cattle. *Fırat Üniversitesi Sağlık Bilimleri Dergisi*, 15(1),139-144.
- Hatipoğlu, F., Özdemir, O., & Kiran, M.M. (2009).** Detection of argyrophil nucleolar organizer regions (AgNORs) and proliferating cell nuclear antigen (PCNA) in epithelial skin tumours from domestic animals. *Revue Médecine Vétérinaire*, 160(10), 477-83.
- Hepelman, M., Strüve, K., Hansmann, F., Seehusen, F., & Kehler, W. (2019).** Urethral fistula after resection of a penile fibropapilloma in a Holstein Friesian bull. *Schweizer Archiv Fur Tierheilkunde*, 161, 553-557.
- Sedano, M., Ramos, E., Choudhari, R., Harrison, A., Subramani, R., Lakshmanaswamy, R., Zilaie, M., & Gadad, S.S. (2020).** Hypoxanthine phosphoribosyl transferase 1 is upregulated, predicts clinical outcome and controls gene expression in breast cancer. *Cancers (Basel)*, 12 (6), 1522.
- Jelínek, F., & Tachezy, R. (2005).** Cutaneous papillomatosis in cattle. *Journal of Comparative Pathology*, 132(1), 70-81.
- Kamiloğlu, A., Öztürk, S., Kılıç, E., & Aksoy, Ö. (2004).** Management and penis and preputium disorders in bulls: 150 cases (2000-2003). *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 10(1), 31-36.
- Kaya, M., Okumuş, Z., Doğan, E., Yanmaz, L.E., & Çetin, E.M. (2010).** Evaluation of penis and preputium diseases in bulls. *Fırat Üniversitesi Sağlık Bilimleri Dergisi*, 24(3), 149-156.
- Kelman, Z. (1997).** PCNA structure, functions, and interactions. *Oncogene* 14, 629-640.
- Khodakaram, T.A., & Kargar, M. (2009).** Gross and histopathologic characteristics of penile fibropapillomas in young bulls. *Comparative Clinical Pathology*, 18,2 61–263.
- Maeda, Y., Shibahara, T., Wada, Y., Kadota, K., Kanno, T., Uchida, I., & Hatama, S. (2007).** An outbreak of teat papillomatosis in cattle caused by bovine papilloma virus (BPV) type 6 and unclassified BPVs. *Veterinary Microbiology*, 121, 242-248.
- Maga, G., & Hübscher, U. (2003).** Proliferating cell nuclear antigen (PCNA): a dancer with many partners. *Journal of Cell Science*, 116, 3051-60.
- Özsoy, Ş.Y., Özyıldız, Z., & Güzel, M. (2011).** Clinical, pathological and immuno-histochemical findings of bovine cutaneous papillomatosis. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 58, 161-5.
- Townsend, M.H., Anderson, M.D., Weagel, E.G., Velazquez, E.J., Weber, K.S., Robison, R.A., & O'Neill, K.L. (2017).** Non-small-cell lung cancer cell lines A549 and NCI-H460 express hypoxanthine guanine phosphoribosyltransferase on the plasma membrane. *OncoTargets and Therapy*. 10, 1921-32.
- Townsend, M.H., Bennion, K.B., Bitter, E.E., Felsted, A.M., Robison, R.A., & O'Neill, K.L. (2021).** Overexpression and surface localization of HPRT in prostate cancer provides a potential target for cancer specific antibody mediated cellular cytotoxicity. *Experimental Cell Research*. 403 (1), 112567.
- Townsend, M.H., Felsted, A.M., Ence, Z.E., Piccolo, S.R., Robison, R.A., & O'Neill, K.L. (2019).** Falling from grace: HPRT is not suitable as an endogenous control for cancer-related studies. *Molecular & Cellular Oncology*, 6(2), 1575691.
- Townsend, M.H., Robison, R.A., & O'Neill, K.L. (2018).** A review of HPRT and its emerging role in cancer. *Medical Oncology*, 35(6), 89.
- Wang, L., Wang, Y., Han, N., Wang, X., & Ruan, M. (2021).** HPRT promotes proliferation and metastasis in head and neck squamous cell carcinoma through direct interaction with STAT3. *Experimental Cell Research*, 399(1), 112424.