RESEARCH ARTICLE Indian Journal of Animal Research, Volume 59 Issue 5: 828-837 (May 2025)

Pathology and Diagnosis of Squamous Cell Carcinoma in Bovines

Vivek Kumar[1], Dhananjay Kumar Jolhe[1], Ratan Chandra Ghosh[1], Prashant M. Sonkusale[2] 10.18805/IJAR.B-5519

ABSTRACT

Background: A total of 20 tissue samples from horn, eye, skin and vulva in bovines suspected of squamous cell carcinoma (SCC) on the basis of gross pathology were collected from Durg, Dhamtari and Rajnandgaon districts of Chhattisgarh and processed in the Department of Veterinary Pathology, College of Veterinary Science and A.H., DSVCKV, Anjora, Durg (C.G.).

Methods: Investigation was conducted during January to June 2022, we explicate the pathological and immunohistochemical examination including scoring and grading of squamous cell carcinoma in bovines. Histopathological examination as per standard HandE method of staining and immunohistochemistry was performed through Benchmark automated staining system.

Result: Twelve cases were diagnosed as SCC on the basis of histopathological and eleven on the basis of immunohistochemical analysis. Grossly neoplasms of horn revealed large, cauliflower like unilateral irregular masses at the base of horn and they were friable and soft in consistency. Small, unilateral growths observed in eye varied from soft to hard in consistency. Histopathological findings in SCC of horn revealed keratinization of horn epithelium forming concentric keratin pearls. Distinctive keratin pearls with more keratin deposition towards the center, numerous mitotic figures with anaplasia and neovascularization were evident in OSCC. Immunohistochemistry revealed strong immunohistochemical expression of Pan-Cytokeratin (Pan-CK), p53 and EGFR and negative to p16. Immunohistochemical scoring (IHS) was done in Pan-CK, EGFR and p53, ranged from 1-4. Well differentiated SCC of horn revealed strong immunoexpression against EGFR, varied from mild to intense. SCC of horn exhibited strong immunoexpression for Pan-CK, p53 and EGFR whereas, immunoexpression in OSCC specifically p53 was less as compared to SCC of horn. Grading and differentiation of SCC (horn and eye) was also done on basis of histopathological and immunohistochemical scoring.

Key words: Egfr, Histopathology, Immunohistochemical Scoring, P16, P53, Pancytokeratin, Papaniculoau.

INTRODUCTION

Squamous Cell Carcinoma (SCC) is one of the most common malignant neoplasm capable of metastatic spread and is observed in various forms across many animals and humans (Yan *et al.,* 2011). The accumulation of genetic and epigenetic alterations (stable and heritable alterations in gene expression and cellular function without changes to original DNA sequence) in cancer cells provides them with unwanted proliferative and metastatic potential. SCC is a malignant tumour of the stratified squamous epithelium of either the skin or a mucous membrane. In bovines most common sites of occurrence of SCC are horn, eye and skin epithelium. SCCs may be of either differentiated or undifferentiated type classified on the basis of histopathology; well differentiated SCCs are characterized by islands of stratified epithelium in dermis having cell nests in the centre and peripheral connective tissue. Undifferentiated SCCs are characterized by group of epithelial cells as cords / islands / trabeculae without keratinized layer in center (Sharma *et al.,* 2020). Squamous cell carcinoma of horn and eye are most commonly seen in bovines and this study is focused on these two forms of squamous cell carcinoma. SCCs are also found at other sites such as skin, ear, vulva, vagina *etc.*

Squamous cell carcinoma of horn or horn core carcinoma, also known as horn cancer, is a significant cancer in cattle mainly affecting indigenous speceies (*Bos indicus)*. It is mostly unilateral and is observed in cattle between 5-10 years of age (Tyagi and Singh, 2006). The bullocks are highly susceptible when compared with bulls and cows. Grossly, it is a neoplastic growth observed with spongy texture, pink cauliflower like tumour having rough and verrucous (wart like) surface which is friable and can bleed easily (Reddy *et al.,* 2017). Histopathologically, SCC of horn is either well differentiated with presence of cell nests (keratin pearls) or poorly differentiated with absence of

[1]Department of Veterinary Pathology, Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya, Anjora, Durg-491 001, Chhattisgarh, India.

[2]Department of Veterinary Pathology, Nagpur Veterinary College, Maharashtra Animal and Fisheries Science University, Nagpur-440 007, Maharashtra, India.

Corresponding Author: Vivek Kumar, Department of Veterinary Pathology, Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya, Anjora, Durg-491 001, Chhattisgarh, India.
Email: vk561997@gmail.com
ORCID: 0009-0002-8349-3084

How to cite this article: Kumar, V., Jolhe, D.K., Ghosh, R.C. and Sonkusale, P.M. (2025). Pathology and Diagnosis of Squamous Cell Carcinoma in Bovines. Indian Journal of Animal Research. 59(5): 828-837. doi: 10.18805/IJAR.B-5519.

Submitted: 07-11-2024 **Accepted:** 07-02-2025 **Online:** 03-04-2025

keratin pearls which are typical concentric layers of keratin forming "epithelial pearls" were observed (Kumar *et al*., 2023; Giri *et al.,* 2011). Some histopathological studies were suggestive of anaplastic changes observed in tumour cells such as hyperchromatic nuclei and mitotic figures. Ocular form of SCC is known as ocular squamous cell carcinoma (OSCC), It is one of the most common neoplasms of eye in bovines. It is the most common tumour afflicting the bovine eye, as well as the most frequently diagnosed cancerous tumour in bovines. The malignant tendencies of this neoplasm makes its early recognition critical. Various biomarkers were used for diagnosis of squamous cell carcinoma (both horn and eye) in the studies conducted in the past, such as: p53, keratins, cytokeratins (pancytokeratins), p16, Epidermal Growth Factor Receptor (EGFR), Vascular Endothelial Growth Factor (VEGF), interleukins *etc*. were detected by immunohistochemistry.

The study was undertaken with the following objectives:

- To study the gross and histopathology of squamous cell carcinoma in affected bovines.
- Detection of squamous cell carcinoma through tumour biomarkers (Pan-CK, p53 gene, EGFR and p16 gene) by immunohistochemistry.

MATERIALS AND METHODS

The study was conducted in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya, Anjora, Durg (Chhattisgarh) to explicate the hematology, gross and histopathological changes and immunohistochemical alterations in squamous cell carcinoma in bovines. The tissues and blood samples were collected from of Durg, Dhamtari and Rajnandgaon districts of Chhattisgarh (Table 3). The study was conducted over a period of 6 months from January to June 2022. Unique case identity (Case ID) was given to each sample. Hematological examination by using Mindray semi auto-haematoanalyser (BC- 2800 Vet). Tissue samples fixed in 10% formalin were processed for histopathological examination and stained as per standard H and E method of staining (Bancroft and Stevens, 1990). Selected tissue samples based on histopathological analysis were processed for immunohistochemistry (IHC).

Immunohistochemistry

Immunohistochemistry was outsourced from Dr Lal PathLabs, PathVets Veterinary Diagnosis, Chittranjan Park, New Delhi. Paraffin embedded tissue sections were deparaffinized and after antigen retrieval, blocking with bovine serum albumin to avoid unwanted binding of antigen and immunolabeling of sections were carried out in automated immunostainers. primary antibodies (Cytokeratin pan-plus, Anti p53, anti- EGFR and Anti- p16) incubation along with immunohistochemical labelling for all markers (p53, p16, Pan-CK and EGFR; Table 1) were carried out on the Bench Mark Automated Staining System (Ventana Medical systems, Inc.), fully automated staining system with 30 individual slide drawers which supports single piece workflow. Antigen retrieval was performed for 60 minutes using Ventana Medical Systems Retrieval

Table 1: Tumour markers and primary antibodies used in immunohistochemical study (Kumar *et al*., 2023).

Tumour markers	Antibody	Clone	Catalogue no.	Make	Lab animal in which Ab raised with Ig class	Volume
Pan-Cytokeratin (Pan-CK)	Cytokeratin Pan plus	AE1 and AE3	MSG098	Zytomed system GmBH, Anhaltiner-stranbe 16, 14163 Berlin, Germany.	N/A	6 ml Conc., RTU
p53	Anti - p53	D07 10M	AM239-	BioGenex Lab., Fremont, California (CA 94538) USA.	Mouse, Ig class: IgG1	10 ml, RTU
Epidermal Growth Factor Receptor (EGFR)	Anti-EGFR	Polycl -onal	AM335-10RE	BioGenex Lab., Fremont, California (CA 94538) USA	Rabbit, N/A	10 ml, RTU
p16	Anti - p16	G175-405	AM540-10 M	BioGenex Lab., Fremont, California (CA 94538) USA	Mouse,Ig class: IgG	10 ml, RTU

RTU: Ready to use, N/A: Not available.

Solution CC1 (according to the method prescribed by Fornazari *et al.* (2017). After addition of enzyme for reaction with DAB (Diaminobenzidine) substrate, slides were counterstained with hematoxylin and examined under light microscope. Immunohistochemical scoring (IHS) of Pan-CK, EGFR and p53 and p16 markers was done in range of 1-4 as per the method of Baghla *et al.,* (2012) (Table 2).

RESULTS AND DSICUSSION

A total of twelve tissue samples were confirmed as SCC (horn and eye) on the basis of histopathology and eleven of them were confirmatory for SCC in Immunohistochemical examination (Table 4). Kalim *et al.* (2021) reported 30 cases of horn cancer out of which 21 cases (67%) were of non-descript breed. The findings of the present study were in accordance with Kalim *et al.* (2021). High occurrence of horn cancer in non-descript animals could be due to more population of non- descript animals in Chhattisgarh state. Kalim *et al.* (2021) reported all 30 clinical cases of horn cancer only in bullocks however this observation was contradictory to our results shown in Table 3.

Haematological examination

The observation of mean±SE values of Haemoglobin (Hb), packed cell volume (PCV), Total erythrocytes count (TEC) and Total leukocyte count (TLC) were estimated (Table 5). The results revealed no significant hematological alterations were observed in bovines affected with squamous cell carcinoma, although in cases of horn as well as ocular cancer, neutrophilia (>60%,) was observed, abundant lymphocytes (45%) were seen in neoplasm of horn and abnormal values of monocytes was observed which may be due to bacteremia, hemolysis and hemorrhage observed. Higher mean value for neutrophils and monocytes were observed in tumour affected bovines in comparison with healthy bovines.

Findings such as neutrophilia was in accordance with Gomes *et al*. (2012). Eosinophilia was reported by Somvanshi (1991) in advance cases of horn cancer but in our study eosinophilia was not observed in any case of neoplasm of horn and eye.

Gross changes

A total of 12 unilateral neoplastic growths suspected to have SCC at horn were examined grossly, which revealed large, irregular masses at the base of horn. Cauliflower like growth at the base of horn were observed in most of the cases. Two cases of large cauliflower like mass of about 20 cm diameter were seen (Fig 1,2). Ocular neoplasms were small, pinkish neoplastic growths, varied from soft to hard in consistency. Large firm, cauliflower like mass, extending deep into the frontal and nasal sinus with verrucous surface was seen in left eye (Fig 3), tumourous

Table 2: Basis for immunohistochemical scoring of tumour cells (Baghla *et al.,* 2012).

Result	Score
Non immunoreactive	0
Immunoreactive in 1-25% cells	1+
Immunoreactive in 26-50% cells	2+
Immunoreactive in 51-75% cells	3+
Immunoreactive in 76-100% cells	4+

Table 3: Details of samples collected (Total number of samples: n=20).

Species	Breed	Sex	Age	Site of growth	District
Cattle	Kosli	F	7 years	Right Horn	Dhamtari
Cattle	Haryana	F	6 years	Right Horn	Dhamtari
Cattle	Non-descript	F	3 years	Right Eye	Durg
Cattle	Holstein Friesian	F	5 years	Right Eye	Durg
Cattle	Holstein Friesian	F	7 years	Right Eye	Rajnandgaon
Cattle	Non-descript	F	6 years	Vulva	Durg
Cattle	Non-descript	F	4 years	Left Horn	Durg
Cattle	Non-descript	M	10 years	Left Horn	Dhamtari
Cattle	Holstein Friesian	F	2 years	Skin (face,neck, shoulder and back)	Durg
Cattle	Kosli	F	2 years	Left Eye	Durg
Cattle	Non- descript	M	9 years	Right Horn	Durg
Cattle	Kosli	F	5 years	Left Eye	Durg
Cattle	Non-descript	M	8 years	Right Horn	Durg
Cattle	Haryana	M	7 years	Right Horn	Dhamtari
Cattle	Non-descript	F	5 years	Left Horn	Rajnandgaon
Cattle	Kosli	M	11 years	Right Horn	Rajnandgaon
Cattle	Non-descript	F	8 years	Right Horn	Durg
Cattle	Non-descript	F	10 years	Left Horn	Durg
Cattle	Non-descript	M	5 years	Right Horn	Durg
Buffalo	Murrah	F	4 years	Left Eye	Durg

mass at medial canthus of right eye extending towards limbus region. One case of neoplastic growth at vulva large cylindrical mass and hard in consistency about 10 cm in length attached proximally to the vulvar lips (Fig 4) and extended to perineal region.

Gross findings were in accordance with the observations of Sharma *et al.* (2020); Fornazari *et al.* (2017) and Pugliese *et al.* (2014). Gross pathological findings of neoplasm of horn was found to be unilateral in accordance with Kalim *et al.* (2021). Pink Cauliflower like soft, friable growth with bleeding at the base of the horn were also reported by Giri *et al.* (2011); Kumar *et al.* (2013) and Sharma *et al.* (2020). Verrucous surface of tumourous growth in eye was reported by Fornazari *et al.* (2017). Tumourous growth observed in vulva (BovVC1) was observed as hard, black cylindrical, ulcerated nodular growth in vulva was also observed by Sreelekshmy *et al.* (2014).

Hisopathological changes

Histopathological findings in SCC of horn were severe keratinization of horn epithelium with concentric keratin pearls also reffered as “cell nests” (Fig 5a). Characteristic epithelial pearls were observed in well differentiated squamous cell carcinoma (Fig 6a and b). More keratin deposition towards the center was prominent (5a andb). Moderately differentiated SCC of horn was characterised with presence of epithelial pearls not as numerous and well defined as compared to well differentiated SCC (Fig 7). Histopathological findings in OSCC were distinctive keratin pearls with more keratin deposition towards the center in BovEC 4 and characteristic epithelial pearls with concentric layers of keratinization in well differentiated OSCC.

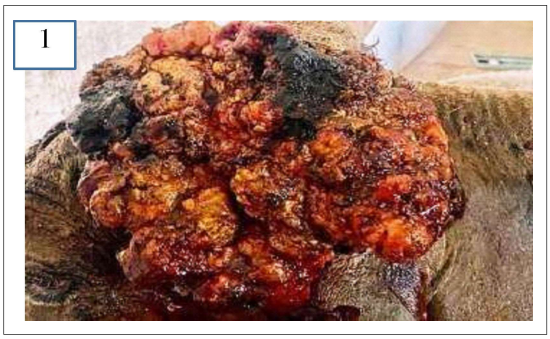


Fig 1: Tumourous mass of about 20 cm diameter from the base of the horn in bullock.



Fig 2: Cut surface of friable tumour and bleeding from the base of the horn in bullock (Case ID: BovHC4) (Kumar *et al.*, 2023).

Table 4: Occurrence of SCC (horn and eye) in Durg, Dhamtari and Rajnandgaon districts of C.G. (on the basis of histopathology and immunohistochemistry).

Site of neopl asm	Number of cases suspected as SCC on the basis of gross pathology	No. of cases confirmed as SCC on the basis of histopathology	No. of cases confirmed as SCC on the basis of immunohistochemistry	Occurrence percentage (On both HP as well as IHC basis)
Horn	12	8	8	66.66%
Eye	6	4	3	50%
Total	18	12	11	61.11%

Note: BovVC1 exhibited the evidence of lipoma in histopathology and non-immunoreactive to p^{53} and Pan-CK in immunohistochemistry whereas BovSC1 histopathology revealed evidence of papilloma (not further processed for IHC).

Table 5: Haematological analysis of bovines suffering from neoplasms of horn, eye, vulva and skin.

Haematological parameters	Observed values (range)	Affected bovine(n=20)	Normal values (range)	Healthy bovine (Control; n=20)
Haemoglobin (g/dl)	7.6-9.6	8.40±0.18	8-15	10.55±0.58
PCV (%)	22.4-35.0	26.20±0.61	25-40	32.50±2.37
TEC (Million/cmm)	7.0-8.0	6.94±0.33	5-9	7.00±0.63
TLC (Thousand/cmm)	6.77-10	8.65±0.29	5-15	8.00±1.26
DLC: Neutrophils (%)	25-62	42.00±3.51	48-55	24±2.84
Lymphocytes (%)	37-45	40.4±0.71	31-35	60±4.74
Monocyte (%)	01-28	9.2±2.47	5-10	5.00±1.58
Eosinophils (%)	00-01	1.00±0.15	2-6	6.00±1.89
Basophils (%)	00-01	0.40±0.12	0-2	1.00±0.31
Platelet count (thousand/cu.mm.)	283-300	285.6±2.442	130-600	488±74.6

Mineralization was also observed around the periphery of keratin pearls (Fig 8). Multiple islands of invasive tumour cells with mononuclear cell infiltration and keratinization at the center of neoplastic islets in moderately differentiated OSCC (Fig 9). Numerous mitotic figures were also noticed with anaplasia and neovascularization (Fig 10). Large tumour islands with mononuclear cell infiltration were seen in moderately differentiated OSCC. Poorly differentiated OSCC is characterized with absence of cell nests. Microscopic examination of vulvar neoplasm revealed lipoma with multiple fat vacuoles and adipocytes tissue separated with thin, inconspicuous fibrous septa (Fig 11 and Fig 12).

Results of microscopic examination were consistent with the findings of Velavan *et al.* (2024); Kalim *et al.* (2021); Sharma *et al.* (2020); Reddy *et al.* (2017); Kumar *et al.* (2013) and Joshi *et al.* (2009). The formation of keratin pearl is due to the lack of cohesion among epithelial cells as a result of malignant changes leading to the concentric arrangement of squamous cells and these cells fate is to form keratin and lay down keratin in concentric manner resembling keratin pearls. Irregular islands of squamous epithelium with cords / islands and clusters of tumourous squamous cells were also reported by Manasa *et al.* (2023); Kalim *et al.* (2021) and Devi *et al.* (2010). Islands of tumourous epithelial cells, proliferation of fibroblasts, connective tissue and infiltration of lymphocytes and plasma cells were also reported in OSCC (Lakshmi *et al*., 2020; Sharma *et al.,* 2020; Fornazari *et al*., 2017 and Islam *et al*., 2017). Invasion of dermal stroma and highlighted keratinization with infiltration of neoplastic squamous cells observed in OSCC were also observed by Fornazari *et al.* (2017). Numerous mitotic figures, anaplasia and neovascularization were in accordance with Velavan *et al.* (2024); Sharma *et al.* (2020); Fornazari *et al.* (2017) and Giri *et al.* (2011).

Immunohistochemistry

Immunoexpression of Pancytokeratin (Pan-CK)

Variations were observed in the immunohistochemical reactivity of Pan-CK between SCC of horn and eye. Immunohistochemical scoring method was mentioned in Table 3. Mostly cases of SCC of horn revealed positive immunoexpression in >75% neoplastic cells and was given score of 4+ (BovHC3; Fig 13), 3+ (BovHC4, BovHC6). High cytoplasmic reactivity of Pan- CK in more than 50% of



Fig 3: Large firm cauliflower like mass, extending deep into the frontal and nasal sinuses, congested with verrucous surface was seen in left eye (Case ID: BovEC4) (Kumar *et al.,* 2023).

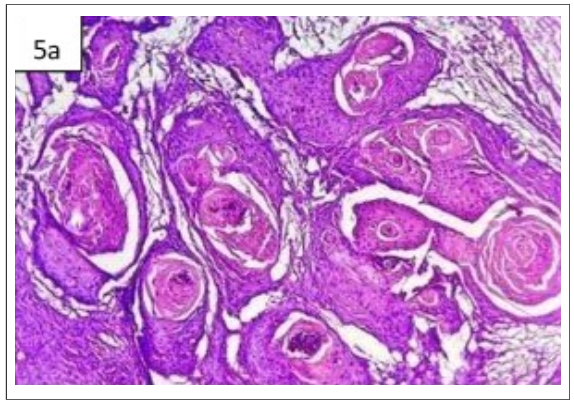


Fig 5a: Excessive keratinization of horn epithelium with concentric arrangement forming keratin pearls/cell nests (Case ID: BovHC6; H and E × 100). (Kumar *et al.,* 2023).



Fig 4: Large hard cylindrical mass about 10 cm in length attached proximally to the vulvar lips and extended to perineal region (BovVC1).

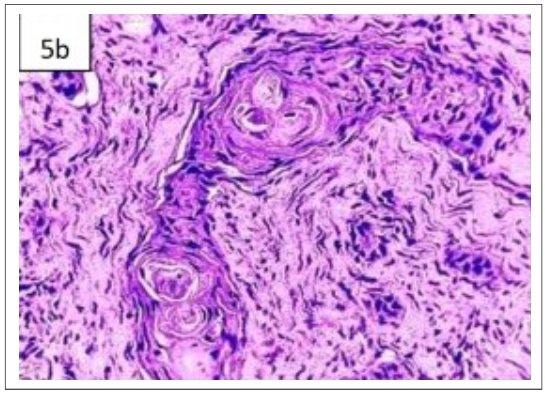


Fig 5b: Well differentiated SCC of horn with keratin pearls and clearly differentiated cyst with fibroblasts and epithelial cells, clusters of tumour cells are visible.

tumourous cells in well differentiated SCC of horn. Pan-CK immunoexpression was more prominent in epithelial pearls in case of well differentiated SCC of horn (Fig 13) and more intensely stained periphery/ border inside large keratin/epithelial pearl (Fig 13), well differentiated SCC of horn depicted strong cytoplasmic staining in almost all neoplastic cells and was given the score of 4+ (Fig 13, 14). Poorly differentiated SCC of horn with absence of keratin pearls/cell nests showed strong staining in about 75% of neoplastic cells, given the score of 3+; Pan-CK immunoexpression in epidermal layer with 2+ (BovHC1). Reddish brown staining of Pan-CK in layered keratinization and tumour islands of BovHC9 and strong Pan-CK immunoexpression inside epithelial pearl scored as 3+ (Table 6) were also found with clear demarcation observed. BovEC4 revealed strong immunoreactivity of Pan-CK in

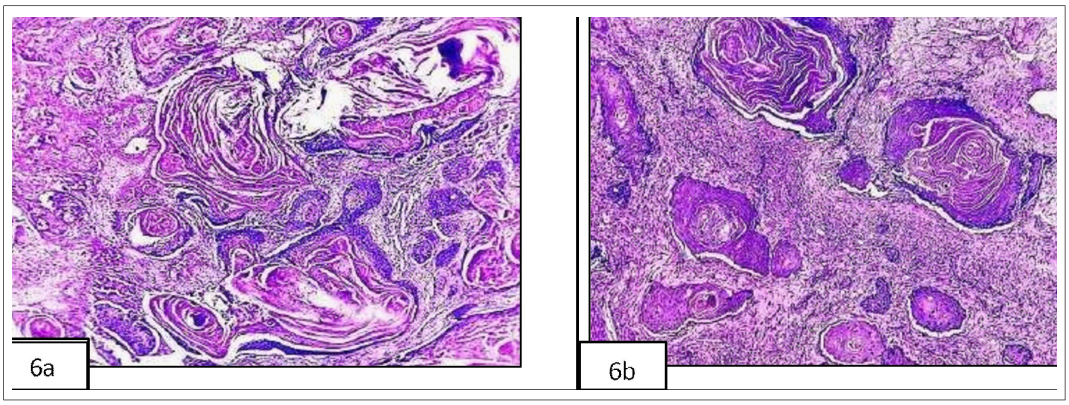


Fig 6 a and b: Multiple keratin pearls with formation of keratin microcyst in well differentiated SCC of horn (BovHC10; H and E × 100).

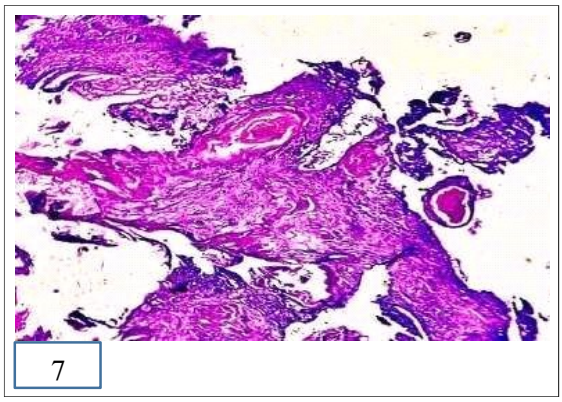


Fig 7: Moderately differentiated SCC of horn with presence of epithelial pearls (BovHC8; H and E ×100).

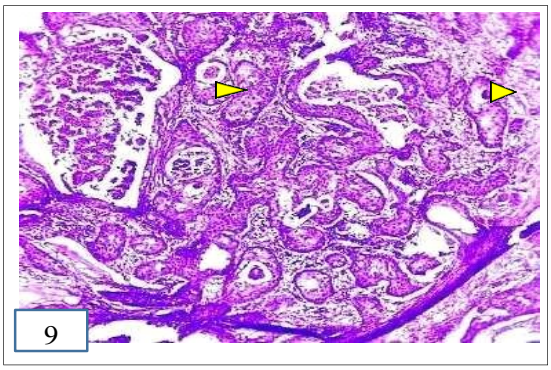


Fig 9: Cords of invasive tumour cells with mononuclear cell infiltration and keratinization at the center of neoplastic islets in OSCC (BovEC3; H and E × 100) (Kumar *et al.*, 2023).

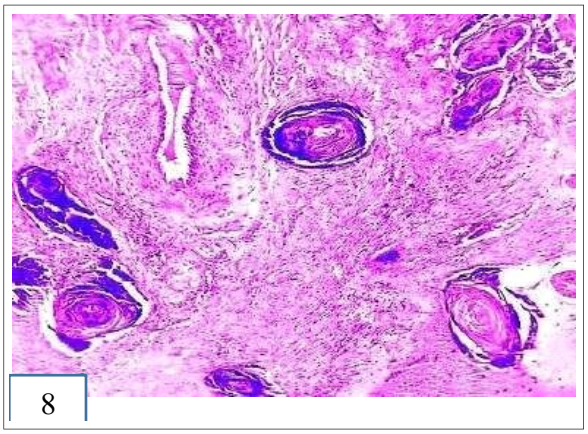


Fig 8: Mineralization around the periphery of keratin pearls (BovEC4; H and E × 100).

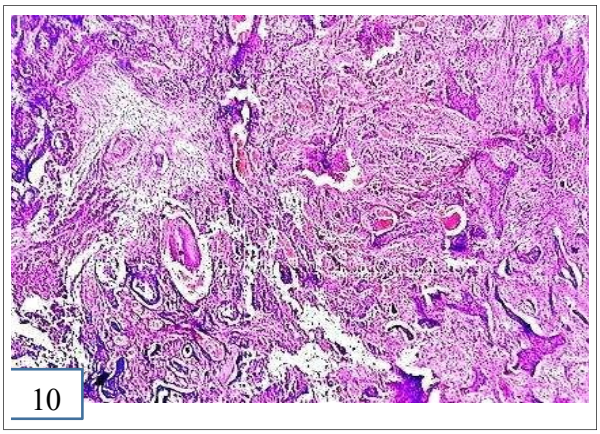


Fig 10: Neovascularization in poorly differentiated OSCC (arrows) (BovEC2; H and E × 40).

the cell nests (keratin pearls) as well as in tumour islands (Fig 17 and 18). Lipoma of vulva exhibited almost no cytoplasmic reactivity for Pan-CK (IHS 0; Fig 21).

Immunoexpression of EGFR

Strong immunoexpression of EGFR with the score of 4+ in well differentiated SCC of horn (Fig 15). Immunoexpression

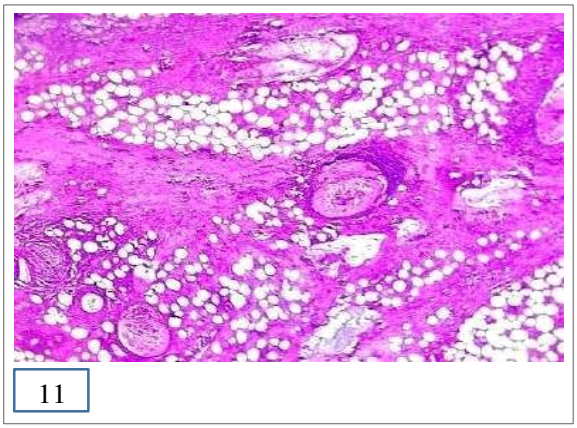


Fig 11: Mass of white adipose tissue separated with thin, inconspicuous fibrous septa in vulva indicating lipoma (BovVC1; H and E × 100).

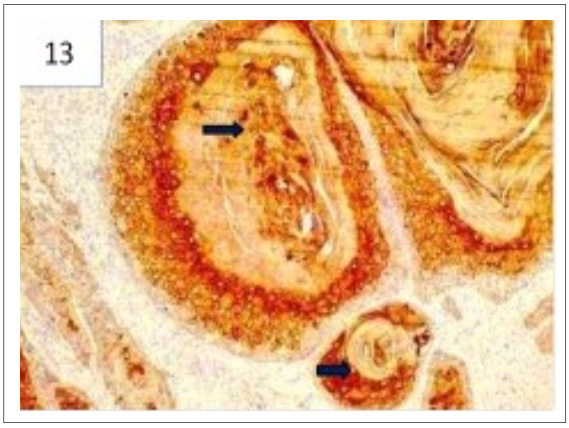


Fig 13: Intense Pancytokeratin immunohistochemical staining inside large keratin pearls (arrow) with the score of 4+ (BovHC3; IHC × 400).

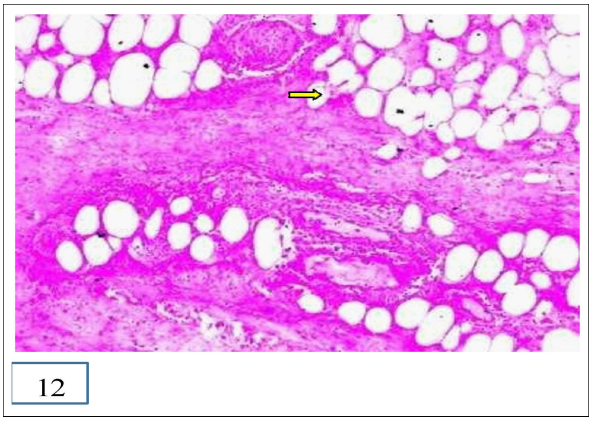


Fig 12: Higher magnification of fig. no. 11 (H and E × 400).

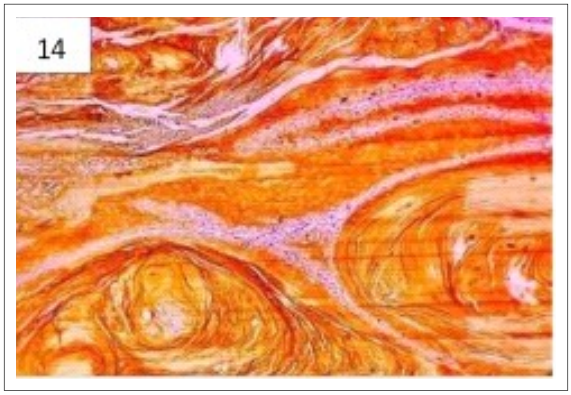


Fig 14: Intense staining of Pan-CK inside keratin pearls leaving the peripheral region around the pearls (arrow) (IHC × 400) (Kumar *et al.*, 2023).

Table 6: Immunohistochemical scoring of different tumour markers for SCC of horn.

Case ID	Hispathological diagnosis	p^{53}	Pan-CK	EGFR	p^{16}
BovHC1	Moderately differentiated SCC	0	2+	0	0
BovHC2	Poorly differentiated SCC	0	1+	0	0
BovHC3	Well differentiated SCC	4+	4+	4+	0
BovHC4	Poorly differentiated SCC	3+	3+	3+	0
BovHC6	Well differentiated SCC	2+	3+	2+	0
BovHC8	Moderately differentiated SCC	1+	1+	0	0
BovHC9	Well differentiated SCC	1+	3+	2+	0
BovHC10	Well differentiated SCC	1+	3+	1+	0
BovEC2	Poorly differentiated SCC	0	0	0	0
BovEC3	Moderately differentiated SCC	2+	3+	3+	0
BovEC4	Well differentiated OSCC	1+	3+	1+	0
BovEC6	Fibrosis with evidence of poorly differentiated OSCC	1+	2+	1+	1+
BovVC1	No evidence of SCC, Lipoma was detected	0	0	Not performed	Not performed

of EGFR was observed in the surrounding tissue excluding large cell nests and in periphery of distinct keratin pearls with absence of immunoexpression inside cell nests (Fig 16). BovHC4 revealed strong immunoexpression with IHS of 3+, which signified its highly malignant and invasive nature even after the absence of well differentiated cell nests. Both SCC of horn and eye showed positive immunoreaction towards EGFR although few samples were detected non-immunoreactive towards EGFR.

Immunoexpression of p53

Tumour suppressor p53 gene immunoexpression was more pronounced in nucleus of cancerous cells in well differentiated SCC of horn. SCC of horn exhibited p53 nuclear staining of tumour cells with a score of 4+ (BovHC3; Fig 19) scattered in connective tissue stroma and peripheral neoplastic cells of tumour islands showing intense nuclear staining of p53. BovEC4 revealed mild to moderate immuno-positive reaction towards p53 with 1+ score . p53 immunore-activity was not observed in lipoma (BovVC1; Fig 22).

Immunoexpression of p16

Negative reaction/non-immunoreactive towards p16 was observed in all SCC of horn including well differentiated (BovHC3; Fig 20), moderately differentiated (BovHC1) and poorly differentiated (BovHC4). BovEC6 exhibited mild immunoexpression for p16 with dark brown staining of nuclei and cytoplasm of tumour cells given the IHS of 1+ (Table 6).

Immunohistochemical findings were partially in accordance with the findings of Sharma *et al.* (2020). They observed Strong cytoplasmic immunoexpression of Pan-CK in almost all neoplastic cells in poorly differentiated SCC but present study revealed highest immunoexpression of Pan-CK in well differentiated SCC of horn (BovHC3) although high and moderate immunoexpression of Pan-CK was observed in poorly and moderately differentiated SCC. Nuclear staining of tumour cells around periphery of epithelial pearls, sparing the region of keratinization with p53 tumour marker was also reported by Carvalho *et al.,* (2005), Fornazari *et al.* (2017) and Sharma *et al.* (2020). High immunoexpression in well differentiated SCC of horn contradicts from the findings of Sharma *et al.,* (2020) where strong immunoexpresssion of p53 was observed in poorly differentiated SCC of horn and OSCC. Intense positive immunoexpression of p53 and expression mostly within outer epithelial layer of the cell nests was observed by Fornazari *et al.* (2017). Findings of immunoexpression of EGFR were similar with Lakshmi *et al.* (2020). The malignancy of tumour of epithelial origin was confirmed by

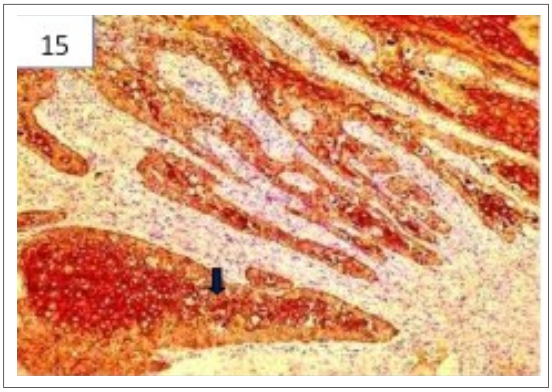


Fig 15: EGFR immunoexpression in islands of SCC of horn (BovHC3; IHC × 100).

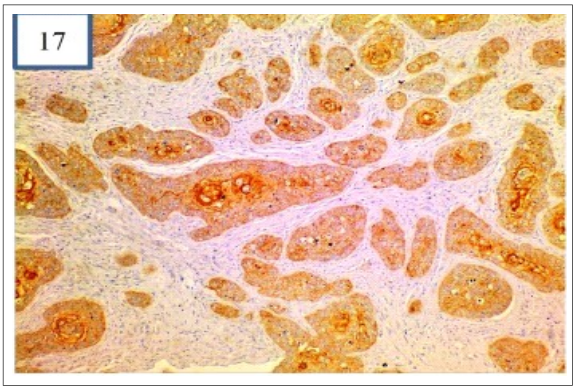


Fig 17: Strong immunoexpression of Pan-CK in well differentiated OSCC (BovEC4; IHC × 100) (Kumar *et al.,* 2023).

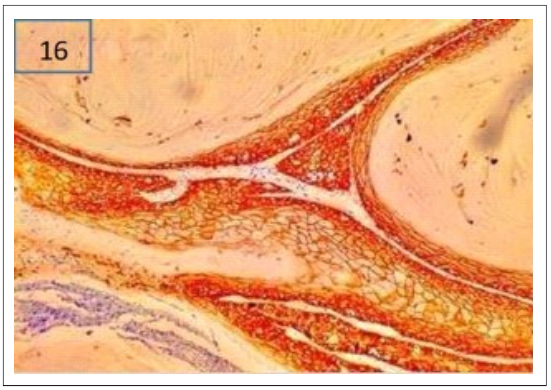


Fig 16: EGFR immunoexpression in the surrounding tissue excluding large keratin pearls (arrows) (BovHC3; IHC × 400) (Kumar *et al.,* 2023).

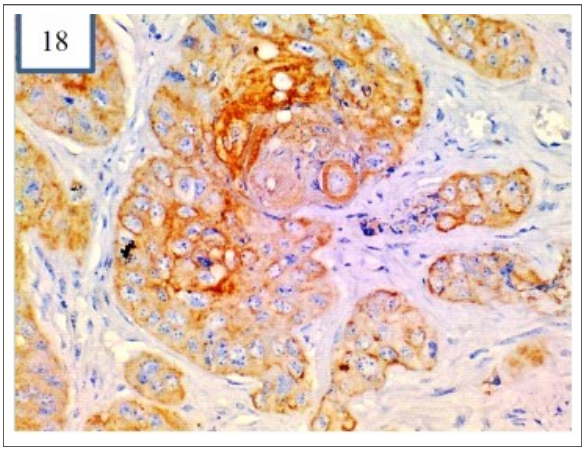


Fig 18: Higher magnification (BovEC4; IHC × 400).

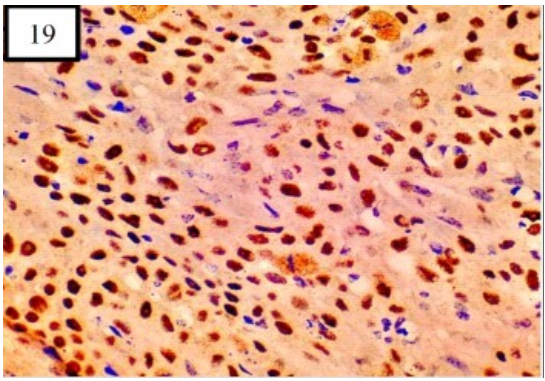


Fig 19: p53 nuclear staining of tumour cells with 4+ score (BovHC3; IHC × 400) (Kumar *et al.,* 2023).

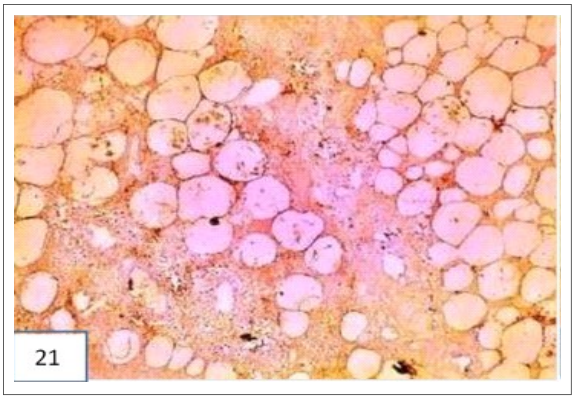


Fig 21: Large vacuolated adipocytes with no immunoexpression of Pan-CK in vulvar lipoma (BovVC1; IHC × 100).

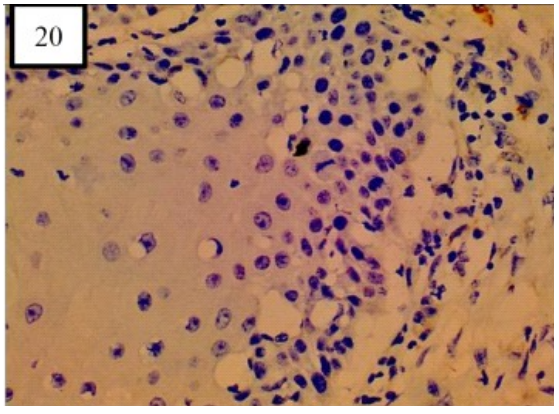


Fig 20: Negative immunoreaction of p16 (arrows) (BovHC3; IHC × 100).

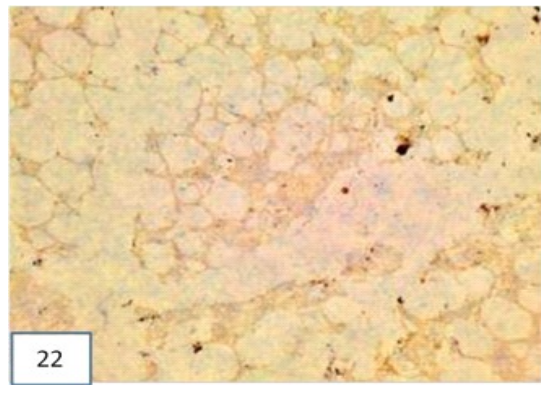


Fig 22: Large vacuolated adipocytes separated by delicate fibrous septa with no immunoexpression of p53 in vulvar lipoma (BovVC1; IHC × 100).

high immunoexpression of EGFR. Findings of p16 immunoexpression were consistent with Fornazari *et al.* (2017) as negative immunoexpression of p16 was reported in majority of cases of OSCC. Marinescu *et al.* (2016) reported positive immunoexpression for p16 in poorly differentiated squamous cell carcinomas in the periphery as well as random in the islands of neoplastic cells. Further investigations are required for p16 immunoexpression as a prognostic factor for bovines with cutaneous SCC as has been previously established for humans with the same tumour in the skin (Marinescu *et al.,* 2016).

Grading and differentiation

Grading of SCC (horn and eye) revealed a total of 3 cases (2 of horn; 1 of eye) as Grade I -poorly differentiated SCC, 3 cases (2 of horn; 1 of eye) as Grade II -moderately differentiated SCC and 5 cases (4 of horn; 1 of eye) as Grade III -well differentiated SCC (Table 7) on the basis of histopathological and immunohistochemical examination.

Table 7: Grading of SCC (horn and eye) on the basis of HP and IHC.

Grade of malignancy	SCC of horn	OSCC	Total
Grade I (Poorly differentiated SCC)	2	1	3
Grade II (Moderately differentiated SCC)	2	1	3
Grade III (Well differentiated SCC)	4	1	5

Conclusion

- ✓ A total of eleven tissue samples were confirmed as SCC (horn and eye) on the basis of both histopathology and Immunohistochemistry.
- ✓ Haematological values did not reveal any significant deviation from the normal values however in fewcases neutrophilia (>60%) and monocytosis was observed.
- ✓ Grossly, tumours suspected of SCC were unilateral, large cauliflower like ulcerated growth bleeding at the base of the horn with rough and verrucous surface.
- ✓ Histopathologically, keratin pearls with high degree of keratinization and layered pattern of keratinization were reported in well differentiated SCC of horn as well as eye with anaplasia, numerous mitotic figures, tumour islands and severe inflammation, neovascularization, hemorrhages *etc.*
- ✓ Lack of cohesion among epithelial cells as a result of malignant changes proceeds to the concentric arrangement of squamous cells forming keratin and lay down keratin

in concentric manner resembling keratin pearls.

- ✓ SCC of horn revealed strong immunohistochemical staining of Pan-CK, p53 and EGFR and negative to p16.
- ✓ In OSCC, Strong, moderate and weak immunoexpression of Pan-CK, EGFR and p53 was observed. The immunoexpression of p53 was less as compared to SCC of horn.

Conflict of interest

All authors declared that there is no conflict of interest.

REFERENCES

Baghla, A., Choudhry, S., Kataria, A. (2012). Immunohistochemical expression of cytokeratin 5/6 in gynaecological tumors. International Journal of Pathology. 13: 1-7.

Bancroft, J.D. and Stevens, A., (Eds.). (1990). Theory and Practice of Histological Techniques. Churchill Livingstone.

Bhatia, D., Hinsu, A., Panchal, K., Sabara, P., Jakhesara, S. and Koringa, P. (2020). Molecular portrait of squamous cell carcinoma of the bovine horn evaluated by high-throughput targeted exome sequencing: A preliminary report. BMC Veterinary Research. 16: 1-7.

Carvalho, T. Vala, H. Pinto, C., Pinho, M., Peleteiro, M.C. (2005). Immunohistochemical studies of epithelial cell proliferation and p53 mutation in bovine ocular squamous cell carcinoma. Veterinary Pathology. 42: 66-73.

Devi, V.R., Veeraiah, G., Annapurna, P. and Estheru, S. (2010). Squamous cell carcinoma of ear in an Indian water buffalo (*Bubalus bubalis*). Brazilian Journal of Veterinary Pathology. 3(1): 60-62.

Fornazari, G.A., Kravetz, J., Kiupel, M., Sledge, D., Filho, IRDB, Montiani-Ferreira, F. (2017). Ocular squamous cell carcinoma in Holstein cows from the south of Brazil. Veterinary World. 10: 1413-1420.

Giri, D.K., Kashyap, D.K., Dewangan, G., Tiwari, S.K., Ghosh, R.C., Sinha, B. (2011). Squamous cell carcinoma of horn and its surgical management - A report of three cases. International Journal of Livestock Research. 1: 55-58.

Gomes, R.G., Rafael, L.A., Headley, S.A. and de Almeida Sampaio, A.J.S. (2012). Clinical and histopathological features of horn core carcinoma in a Nellore cow-Case report. Semina: Ciências Agrárias. 33(5): 1931-1936.

Islam, S.T., Khurma, J., Wani, J.M., Ganaie, M.Y., Singh, A.K., Rashid, H. and Fayaz, I.B. (2017). Ocular squamous cell carcinoma in a female buffalo: A case report. Journal of Entomology and Zoology Studies. 5(6): 795-796.

Joshi, B.P., Soni, P.B., Ferar, D.T., Ghodasara, D.J., Prajapati, K.S. (2009). Epidemiological and pathological aspects of horn cancer in cattle of Gujarat. Indian Journal of Field Veterinarians. 5(2): 65-67.

Kalim, M.O., Tiwari, S.K., Sharda, R., Shakya, S., Sannat, C., Praveen, K., Deshmukh, S.K. (2021). Studies on prevalence, predisposing factors and microscopic evaluation of bovine horn cancer. The Pharma Innovation Journal. 10 (2): 268-270.

Kumar, V., Jolhe, D.K., Ghosh, R.C., Sonkusale, P.M., Sharma, S. (2023). Pathology and diagnosis of ocular squamous cell carcinoma in bovines. Indian Journal of Veterinary Sciences and Biotechnology. 19(4): 43-49.

Kumar, V., Jolhe, D.K., Ghosh, R.C., Dewangan, R., Sonkusale, P.M. and Sharma, S. (2023). Patho-morphological and immuno-histoc hemical studies on bovine horn core carcinoma. Indian Journal of Animal Research. 57(11): 1485-1496. doi: 10.18805/IJAR.B-5206.

Kumar, V., Jolhe, D.K., Ghosh, R.C., Dewangan, R. (2024). Histopathological studies on horn and ocular carcinoma in bovines. Indian journal of Veterinary Pathology. 48(2): 106-115.

Kumar, V., Mathew D.D., Kumar N., Arya, R.S. (2013). Horn cancer in bovines and its management. Intas Polivet 14(1): 56-57.

Lakshmi, M.P., Veena, P., Kumar, R.S. and RaniPrameela, D. (2020). Clinical, pathological and immunohistochemical studies on bovine eye cancer. The Pharma Innovation Journal. 9: 353-355.

Liggett Jr, W.H. and Sidransky, D. (1998). Role of the p16 tumour suppressor gene in cancer. Journal of Clinical Oncology. 16(3): 1197-1206.

Manasa, B., Sujani, G., Babu, K.N., Srinivas, V., Purushotham, C., Nandani, S. and Kumar, R.A. (2023). A case report of mammary carcinoma in royal Bengal Tiger. Bhartiya Krishi Anusandhan Patrika. 38(1): 95-97. doi: 10.18805/BKAP614.

Marinescu, A., Stepan, A.E., Mrgritescu, C.L.A.U.D.I.U., Marinescu, A.M., Zvoi, R.E., Simionescu, C.E. and Niculescu, M. (2016). P53, p16 and Ki67 immunoexpression in cutaneous squamous cell carcinoma and its precursor lesions. Rom J. Morphol. Embryol. 57(2): 691-696.

Pugliese, M., Mazzullo, G., Niutta, P.P., Passantino, A. (2014). Bovine ocular squamous cellular carcinoma: A report of cases from the Caltagirone area, Italy. Veterinarski arhiv. 84(5): 449-457.

Reddy, K.J.M., Kumar, V.G., Ganesh, S., Raghavender, K.B.P. (2017). Unilateral horn cancer in cow and its surgical Management-A case report. International Journal of Current Microbiology and Applied Sciences. 6: 3349-3352.

Sharma, S., Gupta, R.P., Jangir, B.L., Lather, D., Hazari, R. (2020). Pathomorphological studies and immunohistochemical expression of p53 and pancytokeratin in bovine epithelial tumors. Indian Journal of Veterinary Pathology. 44(1): 1-6.

Singh S and Singh G. (2005). Important aspects of horn cancer. Scientific Economical Journal. 2(6): 32-39.

Somvanshi, R. (1991). Horn cancer in Indian cattle. Veterinary Bulletin. 61: 901-911.

Sreelekshmy, M., Tailie, W.A., Pawde, A.M. and Somvanshi, R. (2014). Vulvar Squamous Cell Carcinoma in a Cow: A Case Report.

Taylor, R.L. and Hanks, M.A. (1969). Viral isolations from bovine eye tumors. Am. J. Vet. Res. 30(10): 1885-1856.

Tyagi, R.P.S. and Singh, J. (2006). Ruminant Surgery, 1st edn., CBS Publisher and Distributors. New Delhi. pp. 415-416.

Vala, H., Carvalho, T., Pinto, C., Pereira, M.A., Mesquita, J.R., Peleteiro, M.C. and Fondevila, D. (2020). Immunohistochemical studies of cytokeratins and differentiation markers in bovine ocular squamous cell carcinoma. Veterinary Sciences. 7(2): 70.

Velavan, A., Vijayakumar, M., Kumaresan, A., Mounika, C. and Sugan, S. (2024). Clinical and pathological findings of ulcerative prepuce squamous cell carcinoma in a marwari horse. Indian Journal of Animal Research. 1: 4. doi: 10.18805/IJAR.B-5345.

Yan, W., Wistuba, I.I, Emmert-Buck, M.R., Erickson, H.S. (2011). Squamous cell carcinoma-similarities and differences among anatomical sites. American Journal of Cancer Research. 1(3): 275-300.