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Squamous cell carcinoma in hf crossbred cattle: Clinical features and haematological considerations

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Abstract
Squamous cell carcinoma (SCC) poses a significant threat to Holstein-Friesian (HF) crossbred cattle, impacting both their well-being and agricultural productivity. This study aimed to comprehensively characterize the clinical features of SCC in HF crossbred cattle and evaluate hematological parameters to assess disease progression. A total of 12 HF crossbred cattle diagnosed with SCC were included in the study, with clinical examinations revealing a predominance of ocular and vulvar tumors exhibiting varied gross appearances, ranging from tennis ball-like to cauliflower-like growths. The presence of ulceration and maggot infestation in long-standing cases underscored the chronic nature of the disease. Hematological analyses indicated that parameters remained within normal ranges, despite intralesional cisplatin administration, suggesting limited hematological influence from treatment. These findings highlight the diverse clinical presentations of SCC in HF crossbred cattle and emphasize the importance of implementing comprehensive diagnostic approaches for effective disease management and control in agricultural settings.

Keywords: Squamous cell carcinoma (SCC), cisplatin, intralesional administration, ocular tumours, vulvar tumours

Introduction

In the world of farming, ensuring profitability is paramount, with prudent spending on animal care being pivotal. However, the emergence of squamous cell carcinoma (SCC) in cattle presents a formidable challenge. This condition leads to reduced milk production, increased treatment expenses, and a noticeable decline in the animals' overall health. Consequently, afflicted cattle become more of a burden than an asset for farmers, significantly impacting their livelihoods. In India, where many farmers operate small-scale farms and rely on diversified agriculture for sustenance, the health of dairy cattle is of utmost importance. However, the occurrence of SCC poses a significant threat to their earnings. The higher costs associated with managing and treating SCC cases, coupled with the uncertainty of a complete cure, exacerbate the financial strain on farmers.

Ocular squamous cell carcinoma (OSCC), also known as "cancer eye," is a common and concerning condition among cattle. It develops from epithelial tissues and is the most common malignant tumor in cattle worldwide. Despite its widespread impact, the exact origins of OSCC are unknown, with a variety of factors contributing to its development.

It has been observed that areas of skin lacking hair and pigmentation are particularly vulnerable to developing Squamous Cell Carcinomas (SCC) when exposed to UV radiation (Ramos *et al*., 2007) [24]. Consequently, the vulvar region of the reproductive tract is notably affected by this susceptibility. Additionally, the ocular region is also prone to the development of Squamous Cell Carcinoma.

Research conducted by Den-otter *et al*. (1995) [4] revealed that Simmental cattle with unpigmented periocular skin were at a higher risk of suffering from ocular squamous cell carcinoma (OSCC). Furthermore, Nitya *et al*. (2022) [13] found that ocular cancers were more prevalent in animals raised under a semi-intensive management approach, with access to grazing. Given that many small-scale and marginal farmers in India adopt similar semi-intensive management practices for their cattle, this further increases their susceptibility to SCC.

Therefore, understanding the environmental and management factors contributing to SCC development is crucial for implementing effective preventative measures in cattle farming practices.

Genetic susceptibility emerges as a key factor in OSCC onset, with certain breeds showing increased vulnerability due to complex genetic predispositions. Nutritional imbalances, advancing age, and environmental exposures, particularly ultraviolet (UV) light, all play important roles in the pathogenesis SCC. Notably, circumocular apigmentation, defined as a lack of pigment around the eyes, is a distinct risk factor that increases susceptibility to OSCC.

Despite its widespread impact on various bovine breeds, OSCC has a preference for specific breeds, particularly Hereford, Hereford cross, and Holstein cattle, where it manifests as an invasive and chronically progressive neoplasm.

Contrastingly, in recent years the population of Holstein-Friesian (HF) crossbreed cattle in India has been constantly increasing. And this trend is primarily driven by the desire to enhance milk production efficiency and meet the growing demand for dairy products in the country. HF crossbreeds are favoured for their high milk yields and superior milk quality compared to indigenous cattle breeds. Overall, the rising population of HF crossbreed cattle in India reflects the ongoing transformation of the dairy sector towards more intensive and productive farming practices to meet the growing demand for dairy products in the country. Hence early diagnosis and treatment becomes crucial for the farmer to compensate the economic loss that might occur due to SCC in later stages.

Materials and Methods
The cattle presented with history of chronic swelling or growths were meticulously examined and diagnosed based on gross morphological appearance of growths, nodular growths (Sarangabani et al., 2017; Oral et al., 2019) [27, 14] and tumour cytology (Vara Prasad et al., 2016) [28]. Out of these cases 12 were confirmed for SCC based on FNAC and studied further in detail.

Age, sex, breed, parity, status of pregnancy and site of the tumour, and primary treatment offered, if any, were all thoroughly documented for all the 12 animals. The conjunctival mucous membrane colour and any other irregularities in feeding and watering were also documented. Apart from this the submandibular lymph-nodes were palpated in all the animals having ocular tumours and mammary lymph nodes were palpated in all the cases having vulvar/perineal tumours, for regional swelling if any. Also, the masses were checked for the presence or absence of ulceration, contamination, infection and maggots.

These animals were then surgically operated for excision of tumours and administered with Cisplatin as intralesional adjunct chemotherapeutic agent.

The following physiological parameters were obtained prior to surgery, after surgery and on the twelfth post-operative day in both the groups:
1. Rectal temperature (°F),
2. Respiration rate (breaths/min)
3. Heart rate (beats/min)

In addition, haematological tests were performed for all the animals. Prior to surgery, immediate postoperatively and on 12th post-operative day. The blood was collected from the jugular vein into a K3EDTA (3 ml). Automated haematology analyzer was utilised to test the haematological parameters of the blood taken in the K3EDTA vial.
1. Haemoglobin Estimation (gm/dl)
2. Packed Cell Volume (%)
3. Total Erythrocyte Count (10^6/μl)
4. Total Leukocyte Count (10^3/μl)
5. Differential Leukocyte Count: Neutrophil, eosinophil, monocyte, and lymphocyte differential leukocyte counts were calculated, and the values were reported as percentages.

Results and Discussion
All 12 cattle diagnosed with squamous cell carcinoma (SCC) were adult Holstein-Friesian (HF) crossbred dairy cows, aged between 4 and 10 years, having calved at least once. This age range aligns with observations by Panchabhai et al. (1987) [16] and Pandey et al. (1989) [17], who noted a higher likelihood of SCC occurrence in cattle aged 6–10 years. Cordy (1990) also reported peak incidences of ocular squamous cell carcinoma (OSCC) at 7 and 8 years of age. In terms of eye involvement, among the cases, the left eye was affected in two instances, while the right eye was affected in the remaining cases. Similarly, Ceylan et al. (2012) [2] found a higher prevalence of OSCC in the right eye in their study, contrasting with the observations of Gharagozlou et al. (2007) [8], who reported equal occurrences in both eyes. Nitya et al. (2022) [13] reported a predominance of ocular tumors in the left eye in their study.

Regarding skin pigmentation around the tumors, five cases exhibited white pigmentation, while seven cases showed black pigmentation. Den-otter et al. (1995) [4], however, suggested that cattle with unpigmented periocular skin are more susceptible to OSCC, contradicting our findings, possibly due to the relatively small sample size in our study.

Animals presented for vulvar tumours had relatively larger tumors, there was ulceration in three out of five cases, and maggot infestation in two cases. This aligns with findings by Ozcan-martz et al. (2021) [15] who observed a higher likelihood of perineal/vulvar SCC developing on the mucocutaneous junction compared to the skin. These masses often remain unnoticed in earlier stages due to their small size and location below the tail. Yeruham et al. (1999) [29] recorded the occurrence of vulvar SCC in dairy and beef cattle at 0.91% and 0.38%, respectively. On average, the duration since tumor occurrence and presentation of the cases studied was approximately 3 months.

Table 1: Anamnesis of bovines with SCC (n=12)

Case no.	Age (Years)	Pregnancy	Lactating	Duration of tumour	Location	Skin pigmentation
1	10	Non Pregnant	Yes	Since 2 months	Left Eye	White
2	7	Non-Pregnant	Yes	Since 3 months	Right eye	Black
3	6	Non Pregnant	Yes	Since 2.5 months	Right eye	Black
4	9	Non Pregnant	Yes	Since 1.5 month	Left eye	Black
5	5	Non Pregnant	Yes	Since 3.5 months	Right eye	Black
6	4	6 months Pregnant	No	Since 2 months	Right Eye	Black

7	9	Non Pregnant	Yes	Since 3 months	Right eye	Black
8	8	1 month Pregnant	Yes	Since 6 months	Vulvar tumour	Black
9	8	Non Pregnant	Yes	Since 3.5 months	Vulvar tumour	White
10	10	1.5 month Pregnant	Yes	Since 5 months	Vulvar tumour	White
11	7	Non Pregnant	Yes	Since 3 months	Vulvar Tumour	White
12	6	Non Pregnant	Yes	Since 3 months	Vulvar tumour	White

In the seven cases of ocular squamous cell carcinoma (SCC), six exhibited a tumor with a resemblance to a tennis ball, while one presented a cauliflower-like appearance. Desai (2022) [5] also observed ocular SCC resembling a tennis ball, consistent with the findings of Naik (2010) [28] who noted variations in size from tiny peanuts to large tennis balls. Pugliese et al. (2014) [21] reported three out of twelve cases showing cauliflower-like growth and nine displaying nodular development in ocular SCC.

Similarly, among the five cases of vulvar tumors, three exhibited a nodular gross appearance, while one each resembled cauliflower and lemon. Gharagozlou et al. (2007) [8] found nodular growths to be more prevalent (26/32) than cauliflower-like growths (4/32) in bovines. Conversely, Sarangabani et al. (2017) [27] described the gross shape of vulvar SCC as clinically cauliflower-like. According to Khalil et al. (2020) [10], cutaneous neoplasms in animals can manifest as nodules or indeterminate mass-like developments.

Following gross clinical examination, Fine Needle Aspiration Cytology (FNAC) was performed to confirm the type of tumor, leading to the selection of 12 positive cases for SCC for further treatment procedures. Microscopic examination of these smears revealed characteristics indicative of SCC, such as nuclear pleomorphism, an increased nuclear: cytoplasmic ratio, anisocytosis, and overcrowding of epithelial cells. This aligns with findings by Podarala et al. (2020) [20] who conducted a study on 12 cases of ocular squamous cell carcinoma (OSCC) based on preoperative FNAC results. Similarly, Regmi et al. (2018) [26] encountered a case of SCC in a four-year-old Murrah buffalo, confirmed based on FNAC results showing scattered malignant squamous cells with dense cytoplasm. Mathewos et al. (2020) [12] highlighted the characteristic features evident in FNAC of SCC-positive cases, including irregular nuclear outline, nuclear enlargement, coarse nuclear chromatin, hyperchromasia, and prominent nucleoli.

Table 2: Clinical examination of group-1 bovines with SCC (n=12)

Case no.	Site	Size & Shape	Appearance	Consistency	Margin	Conjuctival mucous membrane
1	L	Cauliflower like	Non ulcerated	Hard	Fused	Pale
2	R	Tennis ball like	Non ulcerated	Hard	Fused	Pink
3	R	Tennis ball like	Non ulcerated	Hard	Fused	Congested
4	L	Tennis ball like	Non ulcerated	Hard	Well circumscribed	Congested
5	R	Tennis ball like	Non ulcerated	Hard	Fused	Pale
6	R	Tennis ball like	Ulcerated and maggot infested	Soft fragile	Well circumscribed	Pale
7	R	Tennis ball like	Non ulcerated	Hard	Fused	Pale
8	Vulva	Large nodular	Maggot infested	Firm	Fused	Pale
9	Vulva	Nodular lemon sized	Ulcerated	Firm	Well demarcated	Pale
10	Vulva	Cauliflower like	Ulcerated and maggot infested	Firm	Well demarcated	Pale
11	Vulva	Nodular	Non ulcerated	Firm	Well demarcated	Pink
12	Vulva	Large nodular	Ulcerated	Firm	Well demarcated	Pale

All 12 animals exhibited signs of good health, including normal feeding, watering, and lactation. According to Gautam et al. (2016) [7], animals affected by SCC maintain alertness and activity levels during feeding. The majority (8 out of 12) had a pale mucous membrane, and it was slightly congested and pink in the remaining two-two cases. This might be attributed to poor nutrition, as the cases have been long-standing.

Upon gross examination, the ocular tumors exhibited varied characteristics. Specifically, six cases presented with a tennis ball-like appearance, while one case displayed a cauliflower-like growth. Additionally, the tumors were assessed for consistency and margins, revealing a spectrum of features such as hardness, firmness, and softness, along with fused or well-demarcated margins. Among these, six out of seven cases demonstrated a hard consistency, with five cases exhibiting fused margins and two cases showing well-circumscribed margins. The majority of tumors in this group (6 out of 7) were non-ulcerated and devoid of maggot infestation.

Notably, Radhakrishnan et al. (1999) [22] documented cases of ulcerated growths with foul-smelling discharge in cattle affected by OSCC. Similarly, Gharagozlou et al. (2007) [8]

reported similar findings of foul-smelling discharge in OSCC-affected cattle.

The majority of vulvar/perineal tumor cases (4 out of 5) presented nodular growths of varying sizes, with one case exhibiting a cauliflower-like appearance. All five tumors displayed a firm consistency with well-demarcated margins, except for one tumor with fused margins. Due to the chronic nature of these cases, with an average duration of approximately 4 months, three out of 5 cases were observed to be ulcerated, with two cases showing maggot infestation.

Prasath et al. (2009) [19] documented a case of cauliflower-like ulcerative vulvar tumor in HF Cross cattle, alongside a hard mass near the vulva with ulceration and crimson red hemorrhages in HF cattle. Similarly, Oral et al. (2019) [14] reported a case of peri-vulvar SCC as a multi-nodular, ulcerated bleeding mass at the right vulvar lip in an eight-year-old HF crossbred cattle. Gautam et al. (2016) [7] and Sarangabani et al. (2017) [27] highlighted maggot infestation as a common sequel of SCC, supporting the findings in this study.

Physiological Parameters

Table 3: Mean ± SE values of physiological parameters in bovines with SCC (n=12)

Intervals	HR (beats/min)	RR (breadth/min)	RT (°F)
Pre operative	78±0.98	16.5±0.8 [a]	100.63±0.37
Post operative	78.92±1.02	15.33±0.9 [a]	100.21±0.26
12th day post operative	76.83±1.53	19.75±1.05 [b]	101.16±0.41
P value	0.477	0.005	0.326

Means bearing different superscripts (a,b) in a row differ significantly ($p<0.05$)
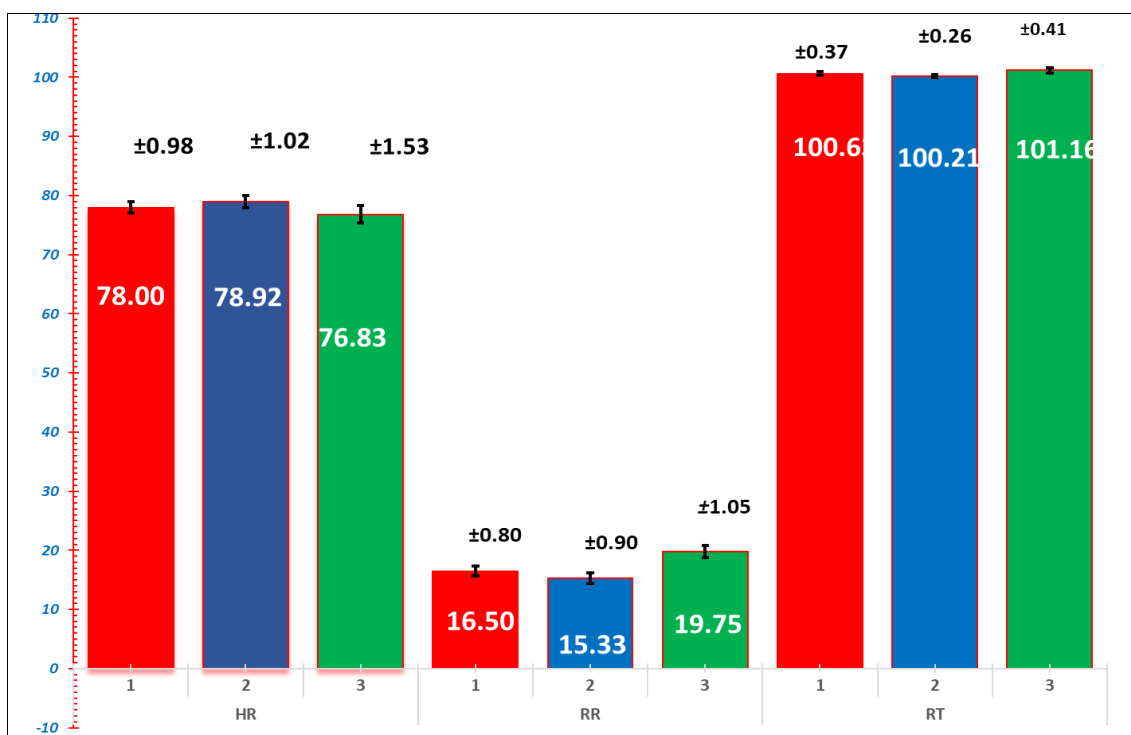
Fig 1: Column graph showing physiological parameters in bovines

Heart rate (beats/minute): The mean values of heart rate on pre-operative, post-operative and on 12th post-operative day were 78±0.98, 78.92±1.02 and 76.83±1.53 respectively.

The calculated p value suggest that no significant variation was observed in mean value of heart rate at different time intervals and the values fluctuated within normal reference range of 60-80 beats/min as mentioned by Jackson and Cockcroft (2002) and Radostits *et al*. (1994) [23].

Swamy (2016) found that mean heart rate values were non-significantly higher on the day before surgery but decreasedto normal after the first postoperative day. Heart rate was within the normal range in a bovine with squamous cell cancer, according to, Gami *et al*. (2017) [6], Sarangabani *et al*. (2017) [27] and Kuma and Sharif (2018) [11] observations.

Respiration rate (breaths/minute)

The mean values of respiration rate in animals on pre operative, post operative and on 12th post operative day were16.5±0.8, 15.33±0.9 and 19.75±1.05 respectively.

Respiratory rate decreased non significantly in the post operative period while significant increase in respiratory rate was noted on 12th post operative day. Furthermore, these fluctuations in respiration were recorded within the normal range of 15-30 beats/min (Jackson and Cockcroft, 2002)

suggesting no distress to the animal prior and post-surgery. Respiratory rates according to Baniadam *et al*. (2010), Gami *et al*. (2017) [6], Islam *et al*. (2017) [9], Reddy *et al*. (2017) [25], Sarangabani *et al*. (2017) [27], Kuma and Sharif (2018) [11] and Regmi *et al*. (2018) [26] remained within normal range for the animals operated for SCC.

Rectal temperature (°F)

The mean values of rectal temperature in animals on pre operative, post operative and on 12th post operative day was 100.63±0.37, 100.21±0.26 and 101.16±0.41 respectively.

There was non-significant variation in mean values of rectal temperature post operatively and non-significant increase was observed at different time intervals. Furthermore, all mean values for rectal temperature were within the normal range of 100.4-102.2 °F as described by Jackson and Cockcroft (2002). These variations were recorded within the normal range suggesting absence of febrile inflammatory reactions both before and after the surgery. Rectal temperature according to Gautam *et al*. (2016) [7], Sarangabani *et al*. (2017) [27], Regmi *et al*. (2018) [26], Desai (2022) [5] remained within normal range for the animals suffering from SCC.

Haematological Parameters

Table 4: Mean ± SE values of haematological parameters in bovine SCC (n=12)

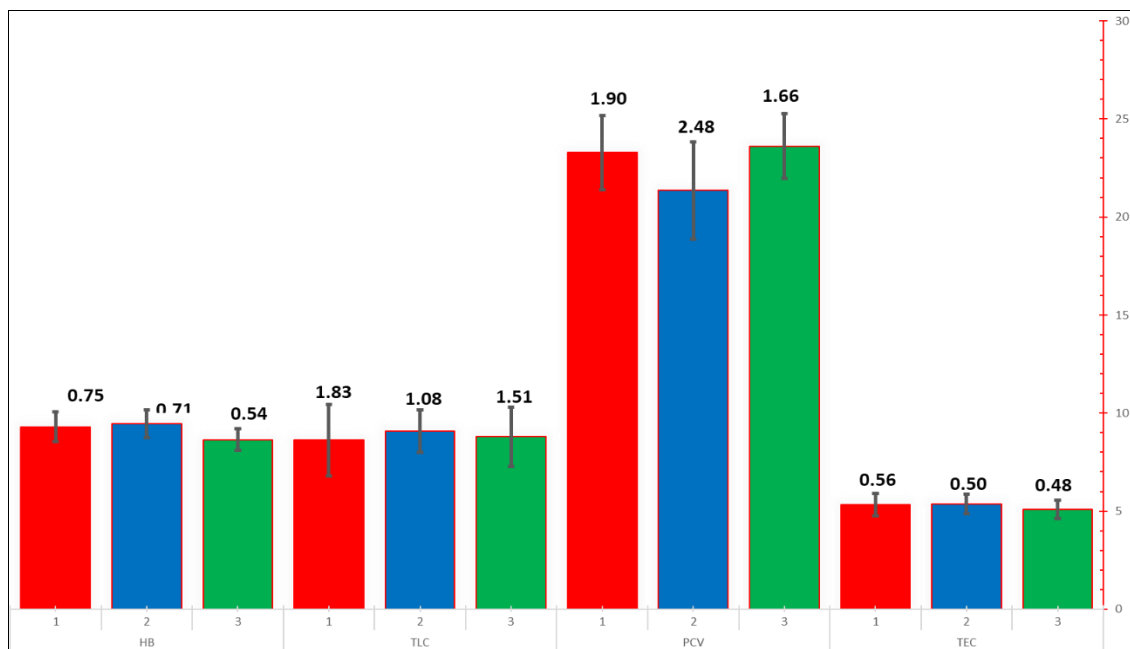| Intervals	HB (gm/dl)	TLC (10^3/μL)	PCV (%)	TEC (10^6/μL)	Platelets (10^3/μL)
0th day pre operative	9.3±0.75	8.62±1.83	23.28±1.9	5.34±0.56	436.25±51.75
0th day post operative	9.47±0.71	9.08±1.08	21.36±2.48	5.37±0.5	496.75±51.29
12th day post operative	8.65±0.54	8.79±1.51	23.61±1.66	5.09±0.48	528.08±77.64
P value	0.668	0.977	0.704	0.919	0.567

Fig 2: Column graph showing haematological parameters in bovines

Haemoglobin (g/dl)
The mean values of haemoglobin on pre operative, post operative and on 12th post operative day was 9.3±0.75, 9.47±0.71 and 8.65±0.54 respectively. There was no significant variation in mean value of haemoglobin at different levels of observations and fluctuations were within normal reference range of 8.0–15 (g/dl) as mentioned by Jackson and Cockcroft (2002) and Radostits *et al*. (1994) [23].

Packed Cell Volume (%)
The mean values of Packed cell volume on pre operative, post operative and on 12th post operative day were 23.28±1.9, 21.36±2.48 and 23.61±1.66 respectively. There was no significant variation in mean value of PCV at different time intervals and values fluctuated within normal reference range of 22-33% as mentioned by Radostits *et al*. (1994) [23] and Constable *et al*. (2017) [3]. Similarly, Gautam *et al*. (2016) [7], Priyanka *et al*. (2021) [20] and Desai (2022) [5] also reported packed cell volume within the normal range in cattle with squamous cell carcinoma. Whereas increase in PCV on 7th postoperative day was observed by, Naik (2010) [28] and Swamy (2016).

Total Erythrocyte Count (10^6/µL)
The mean values of Total Erythrocyte Count on pre operative, post operative and on 12th post operative day were 5.34±0.56, 5.37±0.5 and 5.09±0.48, respectively. The calculated p values suggest that no significant variation was observed in mean value of TEC at different levels of observations and the values fluctuated within normal reference range of 5.1-7.6 (10^6/µL) as mentioned by Radostits *et al*. (1994) [23] and Constable *et al*. (2017) [3].

Total Leukocyte Count (10^3/µL)
The mean values of total Leucocyte count on pre operative, post operative and on 12th post operative day was 8.62±1.83, 9.08±1.08 and 8.79±1.51respectively. The calculated p values suggest that no significant variation was observed in mean value of Total Leucocyte count at different levels of observations and the values fluctuated within normal reference range of 4.9-12 (10^3/mm³) as mentioned by Constable *et al*. (2017) [3].

Platelets (10^3/µL)
In group-1 the mean values of platelets on pre operative, post operative and on 12th post operative day was 436.25±51.75, 496.75±51.29 and 528.08±77.64respectively. The calculated p values for day comparisons suggested no significant variation in mean value of platelets at different levels of observations. These values fluctuated within normal reference range as mentioned by Jackson and Cockcroft (2002) and Radostits *et al*. (1994) [23].

Differential Leukocyte Count
Lymphocyte (%)
The mean values of lymphocytes in animals of group-1 on pre operative, post operative and on 12th post operative day were 35.63±4.09, 28.39±2.91 and 67.98±20.24respectively.
There was no significant variation in mean lymphocyte values calculated for different time intervals.

Monocyte (%)
The mean values of monocytes on pre operative, post operative and on 12th post operative day was 9.68±0.7, 8.99±0.95 and 7.48±0.36 respectively. The calculated p values for different time intervals suggest that no significant variation was observed in mean value of monocytes and the values fluctuated within normal reference range of 2-8% as mentioned by Gautam *et al*. (2016) [7], Priyanka *et al*. (2021) [20] and Desai (2022) [5]. Whereas, reduction in mean values of monocytes at 15th postoperative day was noted by Podarala *et al*. (2020) [20].

Neutrophil (%)
The mean values of neutrophils on pre-operative, post operative and on 12th post operative day were 51.75±4.21 [ab], 59.96±2.98 [a] and 45.24±2.9 [a] respectively.
There was a significant difference observed in the percentage of neutrophils between the pre-operative, post-operative, and 12th day post-operative periods ($p<0.05$), Specifically, there was an initial increase in neutrophil percentage after surgery, followed by a subsequent decrease towards the 12th day post-operative period. This fluctuation suggests a dynamic response of the immune system to the surgical intervention. This fluctuation suggests a dynamic response of the immune

system to the surgical intervention, otherwise the mean value of neutrophils varied within the normal range of 15-45% as reported by Brar *et al*. (2000) [1]. This suggested that the surgery did not significantly affect the neutrophil count, as it remained within the normal range. Gautam *et al*. (2016) [7] observed non-significant differences in neutrophils within the normal range. Priyanka *et al*. (2021) [20] and Desai (2022) [5] also observed non-significant differences in neutrophils within the normal range. Similarly, their study did not find any significant alterations in neutrophil levels in cattle operated for squamous cell carcinoma when compared to the established normal range. These findings collectively suggest that the surgery for squamous cell carcinoma in cattle did not have a significant impact on neutrophil levels, as the neutrophil counts remained within the expected normal range, as reported by the mentioned studies.

Table 5: Mean ± SE values of haematological parameters in bovine SCC (n=12)

Intervals	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)
0th day pre operative	35.63±4.09	9.68±0.7	51.75±4.21 [ab]
0th day post operative	28.39±2.91	8.99±0.95	59.96±2.98 [a]
12th day post operative	67.98±20.24	7.48±0.36	45.24±2.9 [a]
p-value	0.060	0.097	0.016

Conclusions
This research findings highlight a higher incidence of squamous cell carcinoma (SCC) in Holstein-Friesian (HF) crossbred cattle aged around 7 years, with the age range of 4 to 10 years identified as critical for SCC occurrence. Variable tumor localization was observed in the eyes, while the presentation of vulvar tumors with larger sizes, ulceration, and maggot infestation underscores challenges in early detection and management. Additionally, intralesional administration of Cisplatin had no significant effect on hematological parameters, which fluctuated within the normal range.

The study further elucidated the varied gross appearances of ocular and vulvar SCC, ranging from tennis ball-like to cauliflower-like growths. These distinct visual characteristics provide valuable diagnostic cues, aiding in the identification and classification of SCC lesions. The prevalence of pale mucous membranes among the majority of cases suggests potential nutritional inadequacies contributing to the disease.

Overall, the research underscores the diverse clinical presentations of SCC in bovine patients, with distinct characteristics observed in ocular and vulvar/perineal tumors. Gross examination revealed varied tumor appearances, alongside differences in consistency and margins, with ulceration and maggot infestation common in long-standing cases, emphasizing the chronic nature of SCC in affected animals. These findings highlight the importance of comprehensive diagnostic approaches and targeted management strategies for effective disease control in cattle populations.

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