

Case Report

Malignant melanoma in a lamb: clinical and pathological findings

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Abstract

Melanoma is a rare neoplasm in small ruminants, particularly in sheep, and is uncommon in young animals. This study describes the clinical, cytological, histopathological, and immunohistochemical findings of a melanoma in a lamb. A three-month-old female sheep presented with a progressively growing mass in the left periauricular region, first observed at 45 days of age.

27 Cytological evaluation revealed atypical cellular proliferation with marked pleomorphism and mitotic
28 figures, suggesting a malignant neoplastic process. Imaging examinations demonstrated increased
29 soft-tissue volume without evidence of bone involvement. Surgical excision was performed for both
30 diagnostic and therapeutic purposes. Histopathological examination revealed a non-encapsulated,
31 densely cellular, infiltrative neoplasm predominantly composed of epithelioid cells, with marked
32 anisocytosis and anisokaryosis, a high mitotic index, intracytoplasmic melanin pigment, and areas of
33 necrosis. Immunohistochemical analysis showed positive immunostaining for S100 and Melan-A,
34 confirming the melanocytic origin of the neoplasm. Despite surgical removal, rapid local recurrence
35 and clinical deterioration were observed, culminating in death within 30 days, indicating aggressive
36 biological behavior and possible metastatic dissemination. This report demonstrates that, although
37 rare in sheep, melanoma can occur in young animals and may progress rapidly, with a poor prognosis,
38 highlighting the importance of an integrated diagnostic approach and its inclusion in the differential
39 diagnosis of cutaneous masses in lambs.

40

41 **Keywords:** cytology, histopathology, immunohistochemistry, sheep, melanocytes, neoplasia.

42

43 **Introduction**

44

45 Melanocytic neoplasms are tumors derived from the neoplastic transformation of
46 melanocytes, cells originating from the neural crest and distributed in the skin, mucous membranes,
47 eyes, and meninges (13). In domestic animals, melanoma varies among species, being frequently
48 described in dogs and horses, but considered uncommon in ruminants, particularly in sheep (4, 14).
49 In horses, melanomas are particularly common in gray-coated animals, especially aged horses,
50 suggesting that coat pigmentation and melanocytic biology may influence tumor development (11).

51 In small ruminants, these tumors are rare and generally reported as sporadic findings. In
52 Brazil, epidemiological surveys have shown a low incidence of melanoma in sheep, reinforcing its

53 rarity in this species (7). However, when present, these neoplasms may exhibit aggressive biological
54 behavior, with high invasive and metastatic potential (6, 10).

55 The etiopathogenesis of melanoma is multifactorial, involving the interaction between genetic
56 and environmental factors. Ultraviolet radiation is considered a predisposing factor in some species;
57 however, the occurrence of tumors in non-exposed areas suggests the involvement of additional
58 mechanisms independent of solar radiation (14). Furthermore, tumor progression is associated with
59 dysregulation of cellular pathways involved in proliferation, survival, and cell dissemination (3, 14).
60 Recent studies also demonstrate the involvement of molecular pathways such as NF- κ B and TGF- β ,
61 as well as cellular processes including apoptosis and autophagy, which directly influence the
62 proliferative and invasive capacity of tumor cells (16).

63 The clinical and morphological presentation of melanoma in ruminants is heterogeneous,
64 varying in anatomical location, degree of pigmentation, and biological behavior, which may hinder
65 clinical recognition, especially in early stages or in cases with subtle pigmentation (6).

66 Although melanomas are generally more frequent in adult animals, they have also been
67 described in young individuals, including congenital or early-onset forms, suggesting an association
68 with alterations in melanocytic development (2, 4). In cattle, a different pattern is observed, with a
69 higher occurrence in young animals, possibly associated with developmental disorders (1, 9).

70 From an anatomopathological standpoint, melanomas are characterized by the proliferation of
71 epithelioid, spindle-shaped, or mixed cells, with variable degrees of pigmentation, cellular
72 pleomorphism, and mitotic activity, often associated with infiltrative growth and areas of necrosis.
73 Morphological variability, including amelanotic forms, may hinder cytological diagnosis,
74 underscoring the importance of correlating histopathology with immunohistochemistry to confirm
75 melanocytic origin (8, 13).

76 Within this context, this study describes the clinical, cytological, histopathological, and
77 immunohistochemical findings of melanoma in a lamb, contributing to a better understanding of this
78 neoplasm in small ruminants.

79

80 **Case description**

81

82 A female sheep, approximately three months old, mixed breed, with a black coat and body
83 weight of 16 kg, was attended at the Large Animal Clinic of the Department of Veterinary Medicine,
84 Federal Rural University of Pernambuco (AGA/DMV/UFRPE), Brazil. The animal originated from
85 a farm located in the municipality of Moreno (8°07'05" S; 35°05'31" W), in the Metropolitan Region
86 of Recife, Zona da Mata Sul of Pernambuco, Brazil.

87 According to the anamnesis, the animals were fed roughage from *Brachiaria decumbens*,
88 supplemented with hay during dry periods, and a commercial concentrate formulated for sheep. The
89 owner reported that at 45 days of age, the animal developed a swelling at the base of the left ear,
90 initially firm, that progressed toward the frontal bone. An abscess was initially suspected, and
91 drainage was attempted; however, no purulent material was obtained; instead, dark red hemorrhagic
92 fluid was drained. Empirical treatment with dexamethasone and oxytetracycline was instituted, with
93 no clinical improvement and progression of the lesion, which led to referral to the veterinary service
94 at AGA/DMV/UFRPE.

95 On clinical examination, the animal presented a heart rate of 128 bpm, a respiratory rate of 40
96 breaths per minute, and a rectal temperature of 39.8 °C. Cardiac auscultation revealed a regular
97 rhythm without murmurs, and no abnormalities were detected on pulmonary auscultation. Digestive
98 system evaluation showed preserved ruminal motility, with six contractions in five minutes.
99 Inspection of the cranial region revealed an expansile mass measuring approximately 9 cm in
100 diameter, with soft to fluctuant consistency in some areas, covered by intact skin, extending from the
101 left periocular region to the occipital region (Fig. 1A). Hematological evaluation revealed mild
102 microcytic anemia (hematocrit 25% and MCV 26.32 fL), associated with anisocytosis due to
103 microcytosis, as well as hypoproteinemia (5.5 g/dL), mild leukocytosis due to neutrophilia (12.90

104 $\times 10^3/\mu\text{L}$), and mild thrombocytosis ($840 \times 10^3/\mu\text{L}$), with the presence of rare activated platelets and
105 platelet microaggregates.

106 Radiographic evaluation of the skull showed a marked increase in soft-tissue volume in the left
107 periauricular region, with mixed radiopacity and a well-defined expansile appearance, causing
108 displacement of adjacent structures without evidence of bone lysis or proliferation, indicating the
109 absence of bone involvement (Fig. 1B). Ultrasonography suggested content compatible with
110 hematoma or hygroma, with no evidence of abscess formation. Fine-needle aspiration (FNA) was
111 performed, yielding hemorrhagic material, which was submitted for cytological and bacteriological
112 analysis (Fig. 1C).

113 Cytopathological examination revealed a sample with low to moderate cellularity, composed
114 of a mixed lymphocytic population with atypical mesenchymal cells arranged in pseudoaggregates,
115 showing marked pleomorphism, anisocytosis, anisokaryosis, and mitotic figures, including atypical
116 forms. The background contained abundant hemorrhagic fluid containing numerous erythrocytes,
117 macrophages, and cellular debris (Fig. 2A–B). The findings were suggestive of inflammatory
118 infiltrate associated with atypical neoplastic proliferation and necrosis, and histopathological
119 confirmation was recommended. Bacterial culture showed no microbial growth.

120 Based on these findings, surgical excision of the mass was performed (Fig. 1D). The procedure
121 was carried out under general inhalation anesthesia, using a protocol with midazolam, morphine,
122 ketamine, and propofol, with maintenance using isoflurane. An elliptical incision was made with a
123 safety margin of approximately 2 cm, followed by dissection and complete removal of the mass.

124 Macroscopically, the fragments were blackish, irregular, and firm. Microscopically, a non-
125 encapsulated, densely cellular neoplastic proliferation was observed, composed predominantly of
126 pleomorphic epithelioid cells interspersed with fibrovascular stroma. The cells exhibited moderate to
127 abundant, slightly eosinophilic cytoplasm, occasionally containing melanin pigment, and a high
128 nucleus-to-cytoplasm ratio. The nuclei were large, ranging from round to oval, often irregular, with
129 loose chromatin and multiple prominent nucleoli.

130 Marked cellular and nuclear pleomorphism, anisocytosis, anisokaryosis, macrocytosis,
131 macrokaryosis, aberrant nucleoli, as well as nuclear molding and indentation were observed, with the
132 presence of bi- and multinucleated cells. Mitotic activity was high, with 24 mitotic figures in 10 high-
133 power fields (400×), along with multifocal areas of necrosis, mild mineralization, and apoptotic cells
134 (Fig. 2C–D). Immunohistochemical analysis demonstrated positive cytoplasmic and nuclear
135 immunolabeling for S100 and moderate cytoplasmic immunoreactivity for Melan-A in neoplastic
136 cells, confirming the melanocytic origin of the neoplasm (Fig. 2E–F).

137 Postoperatively, the animal initially showed satisfactory recovery under analgesic and
138 antimicrobial therapy. However, within less than 30 days, local recurrence with rapid tumor growth
139 was observed. The animal subsequently developed unilateral left exophthalmos, difficulty in food
140 prehension, and head tilt, progressing to death. Necropsy was recommended but not authorized by
141 the owner, preventing the evaluation of possible metastases.

142

143 **Discussion**

144

145 The present report describes a melanoma in a lamb with aggressive biological behavior,
146 characterized by rapid growth, high mitotic index, early recurrence, and unfavorable clinical outcome.
147 In sheep, this neoplasm is considered rare, especially in Brazil, where epidemiological surveys
148 indicate a low frequency of melanocytic tumors in this species. In this context, descriptions of cases
149 with atypical presentations, such as in young animals, are relevant for expanding current knowledge
150 of the clinical and biological variability of this neoplasm (7).

151 The tumor's occurrence at 45 days of age is one of the most relevant aspects of this case.
152 Although melanomas are more frequently described in adult ruminants, they have also been reported
153 in young individuals, including congenital or early-onset forms, suggesting a possible association
154 with alterations in melanocytic development. Among these, Baker's (2) report describing an invasive
155 melanoma in a newborn lamb stands out as one of the few comparable cases. Similarly, the early

156 onset and rapid progression observed in the present case suggest a highly proliferative biological
157 phenotype, possibly related to alterations in the development or regulation of the melanocytic lineage
158 (2, 4).

159 In the present case, the animal had a black coat, and the lesion developed in a pigmented cranial
160 region. In some species, such as humans, reduced pigmentation and increased exposure to ultraviolet
161 radiation are considered important predisposing factors for melanoma development. Conversely,
162 melanocytic tumors in sheep have also been described in darkly pigmented animals and in anatomical
163 regions not exposed to ultraviolet radiation, suggesting that additional mechanisms independent of
164 solar exposure may be involved in melanocytic transformation (14).

165 The clinical presentation as an expansile mass in the cranial region is consistent with previous
166 descriptions, in which melanomas may occur at different anatomical locations and exhibit wide
167 morphological and behavioral variability. This heterogeneity, including variations in pigmentation
168 and lesion consistency, may hinder clinical diagnosis. In the present case, the initial suspicion of an
169 abscess and the attempt at drainage highlight this diagnostic challenge, as melanocytic lesions may
170 contain hemorrhage and mimic inflammatory processes (6, 8).

171 Fine-needle aspiration cytology revealed a mixed cell population, predominantly lymphocytic
172 with atypical mesenchymal cells, which hindered the initial diagnostic interpretation. This finding
173 reinforces that, although cytology is a useful screening tool, its interpretation may be limited in
174 melanocytic tumors due to high morphological variability, including epithelioid, spindle, and
175 amelanotic forms. Additionally, the presence of a hemorrhagic background and necrosis may
176 compromise cytological evaluation, leading to inconclusive or misleading diagnoses (13). Caseous
177 lymphadenitis was considered among the differential diagnoses due to the anatomical location and
178 the species involved; however, the absence of purulent caseous material, the lack of bacterial growth,
179 and cytological and histopathological findings ruled out this condition (5).

180 Histopathological findings were decisive for diagnosis. The densely cellular neoplastic
181 proliferation, characterized by marked pleomorphism, high mitotic activity (24 mitoses/10 HPF),

182 melanin pigment, and areas of necrosis, supports the diagnosis of malignant melanoma with
183 aggressive behavior. These findings are consistent with reports in small ruminants, in which
184 melanocytic tumors show infiltrative growth and high metastatic potential. The high mitotic index
185 observed in this case further supports the neoplasm's elevated proliferative potential and its
186 association with a poor prognosis (8, 10).

187 Immunohistochemical confirmation of S100 and Melan-A expression was essential to establish
188 the melanocytic origin of the neoplasm, particularly given the observed morphological variability.
189 These markers show high sensitivity for melanocytic tumors and are especially useful in cases with
190 low pigmentation or atypical morphology (13).

191 The clinical course, marked by local recurrence within less than 30 days and rapid progression,
192 supports the aggressive behavior of melanoma. In small ruminants, metastatic dissemination to lymph
193 nodes and visceral organs, such as the liver, lungs, and kidneys, has been reported. In the present
194 case, the absence of necropsy prevented confirmation of metastases; however, the clinical signs
195 observed, including unilateral exophthalmos, head tilt, and difficulty in food prehension, strongly
196 suggest involvement of cranial structures or the central nervous system (10, 12).

197 From a biological standpoint, melanoma aggressiveness is associated with dysregulation of
198 multiple cellular pathways involved in proliferation, survival, and tumor invasion. Recent studies
199 indicate that pathways such as NF- κ B and TGF- β , as well as processes related to apoptosis and
200 autophagy, play a central role in melanoma progression. In melanoma, dysregulation of apoptosis-
201 related pathways may impair programmed cell death, allowing neoplastic cells to survive and expand,
202 while autophagy may contribute to tumor adaptation and progression. These mechanisms may explain
203 the rapid growth, invasiveness, and early recurrence observed in this case, further reinforcing the
204 tumor's highly aggressive nature (16).

205 From a therapeutic perspective, surgical excision remains the main approach; however, it is
206 often not curative in tumors with infiltrative behavior and high proliferative potential. The early

207 recurrence observed reinforces the guarded prognosis of melanoma in small ruminants, especially
208 when diagnosed at advanced stages (12).

209 Thus, the present report highlights that, although rare, melanoma should be included in the
210 differential diagnosis of cutaneous masses in sheep, including in young animals. The integration of
211 clinical, cytological, histopathological, and immunohistochemical evaluation is essential for accurate
212 diagnosis, particularly given the morphological variability and biological behavior of this neoplasm.

213 In conclusion, this report demonstrates that, although rare in sheep, melanoma may occur in
214 young animals and exhibit highly aggressive behavior, characterized by rapid growth, early
215 recurrence, and probable systemic dissemination. Its occurrence in a lamb suggests a possible role of
216 factors related to melanocytic development. The integration of clinical, cytological, histopathological,
217 and immunohistochemical findings was essential for diagnosis, highlighting the importance of a
218 comprehensive approach given the variability of these tumors. The clinical outcome reinforces the
219 guarded prognosis and therapeutic limitations, emphasizing that melanoma should be included in the
220 differential diagnosis of cutaneous masses in sheep, even in young animals, particularly in cases with
221 rapid progression and aggressive behavior.

222

223 **Data Availability**

224 All the original contributions presented in this study are included in the article. Further inquiries
225 can be directed to the corresponding author.

226

227 **Author Contributions**

228 **Márcio Douglas Leal da Silveira:** Conceptualization, Data curation, Formal analysis,
229 Investigation, Resources, Methodology, Writing – original draft. **Maria Luiza de Barros**
230 **Gonçalves:** Investigation, Resources. **Caroline da Silva Penha:** Investigation, Resources. **Camila**
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232 Investigation, Resources. **Huber Rizzo:** Data curation, Visualization, Writing – original draft,

233 Writing – review & editing. **Fabřicio Bezerra de Sa**: Investigation, Resources. **Lucio Esmeraldo**
234 **Honorio de Melo**: Conceptualization, Methodology, Supervision. All authors have read and
235 approved the final version of the manuscript.

236

237 **Conflict of Interest**

238 The authors declare no competing interests.

239

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241 The authors did not use generative artificial intelligence tools or technologies in creating or
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246

247 **References**

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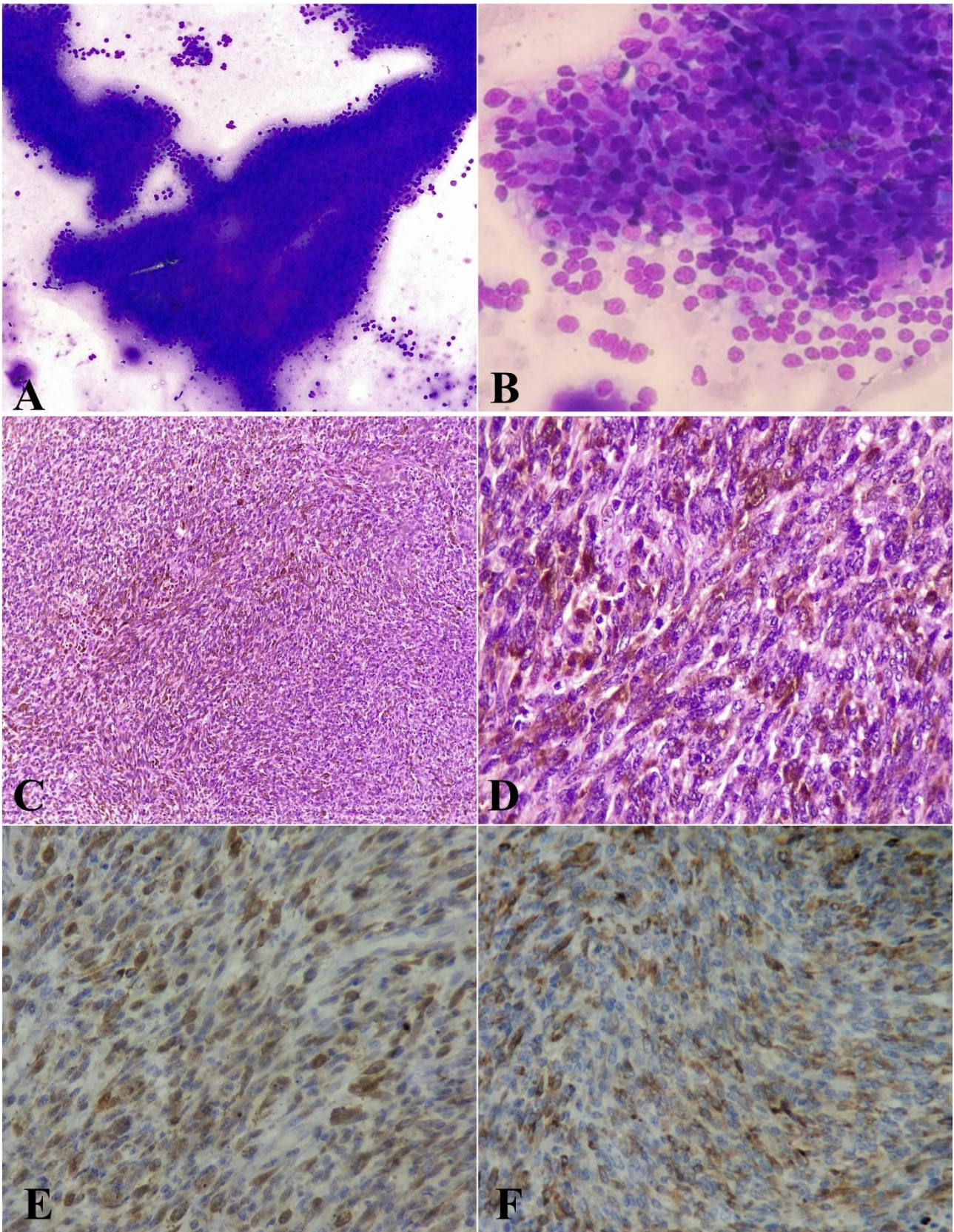
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292

293 **Figure 1.** Clinical and imaging evaluation of melanoma in a lamb. (A) Expansile mass in the left
 294 cranial region, extending from the periocular area to the occipital region, covered by intact skin
 295 (dotted circle). (B) Skull radiographic examination (dorsoventral and lateral projections),
 296 demonstrating marked soft tissue enlargement in the left periauricular region, with mass effect and
 297 displacement of adjacent structures, without evidence of bone lysis or proliferation (arrows). (C) Fine-
 298 needle aspiration (FNA), showing the collection of dark red hemorrhagic fluid. (D) Intraoperative
 299 aspect after surgical excision of the mass.

300



301

302 **Figure 2.** Cytological, histopathological, and immunohistochemical evaluation of melanoma in a
303 lamb. (A) Fine-needle aspiration cytology showing pleomorphic neoplastic proliferation arranged in
304 pseudoaggregates, associated with an eosinophilic matrix, Diff-Quick stain (10×). (B) Cytological

305 detail demonstrating round to oval cells, with indistinct to poorly defined cytoplasm, variable
306 chromatin, and rare pigment granules, Diff-Quick stain (40×). (C) Histopathological examination
307 showing solid proliferation of spindle to epithelioid cells, H&E (10×). (D) Higher magnification
308 demonstrating marked pleomorphism, presence of intracytoplasmic melanin pigment, and atypical
309 mitotic figures, H&E (40×). (E) Positive cytoplasmic and nuclear immunolabeling for S100 in
310 neoplastic cells (40×). (F) Moderate cytoplasmic immunoreactivity for Melan-A in neoplastic cells
311 (40×).