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RESEARCH ARTICLE

Histopathological and Immunohistochemical Insights into Ovarian Tumours in Sheep: A Comprehensive Study

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ABSTRACT

Ovarian tumours, though common in domestic animals, have been sparsely studied in sheep due to their early slaughter upon infertility. This study investigated 212 ovaries collected from 106 sheep to identify and characterize neoplasms. Histopathological and immunohistochemical analyses revealed one sex cord-stromal neoplasm (granulosa cell tumor), three epithelial neoplasms (adenoma, adenocarcinoma, clear cell carcinoma) and four mesenchymal neoplasms (hemangiosarcoma, fibroma, angioleiomyoma, hemangiopericytoma). Granulosa cell tumours were the most prevalent and exhibited distinct histological patterns, including Call-Exner bodies and multifocal coalesced patterns. Adenocarcinoma and cystadenoma presented characteristic growth patterns, while mesenchymal neoplasms exhibited unique histological and immunohistochemical features, such as VEGF positivity in hemangiosarcoma and SMA positivity in angioleiomyoma. These findings align with previous reports in other species, emphasizing the importance of immunohistochemistry in tumor classification and contributing valuable insights into the occurrence and pathology of ovarian tumors in sheep.

Keywords: Histopathological evaluation, immunohistochemistry, ovarian neoplasms, sheep ovaries

INTRODUCTION

Ovarian health is paramount to the reproductive efficiency and overall productivity of domestic animals, directly impacting the profitability of livestock industries. Among the various reproductive disorders, ovarian tumours, though relatively less common than conditions like cystic ovaries, represent a significant pathological concern due to their potential to severely compromise fertility and fecundity across a wide range of species, including cattle, goats, sheep, dogs, cats and mares[1]. While the occurrence of such neoplastic conditions has been documented in veterinary literature, comprehensive and in-depth investigations specifically focusing on ovarian tumours in sheep remain notably limited[2].

Ovarian tumours are classified into three main categories based on their origin: epithelial tumours, sex cord-stromal tumours and mesenchymal tumours. Epithelial tumours, the most frequently observed type, typically arise from the surface epithelium of the ovary, with rare instances originating from the rete ovarii[3,4]. Sex cord-stromal tumours arise from the non-germ cell components of the ovarian stroma and sex cords and include granulosa cell tumours, luteomas, thecomas, Sertoli cell tumours, Leydig cell tumours and roblastomas, arrhenoblastomas and lipid cell tumours[3]. Mesenchymal tumours, though exceedingly rare, include fibromas, hemangiomas, leiomyomas and their malignant counterparts[3,5].

With recent advancements in veterinary pathology, immunohistochemistry (IHC) has become an indispensable tool for diagnosing ovarian tumours. IHC enables precise identification of cellular lineages, differentiation patterns and specific protein markers within neoplastic tissues[6]. These markers help distinguish between tumour subtypes that may appear similar on routine H&E staining and can also reveal the presence of poorly differentiated cells. In the present study, IHC was applied to ovarian samples that had been positively identified with neoplastic changes during histopathological evaluation. Monoclonal antibodies against key markers, including Vascular Endothelial Growth Factor (VEGF), Pan-Cytokeratin and Smooth Muscle Actin (SMA) were used to provide a detailed cellular and structural characterization of ovarian tumours in sheep.

This comprehensive study aims to address existing knowledge gaps regarding ovarian tumours in sheep by first conducting a through histopathological examination to classify these tumours based on their cellular origin and morphological characteristics. Subsequently, it investigates the expression patterns of specific

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immunohistochemical markers across different tumor types, thereby providing detailed cellular and structural insights.

MATERIALS AND METHODS

The present study focused on identifying various ovarian neoplasms in 212 ovaries collected from 106 sheep of different age groups, approximately ranging from two to seven years. The samples were obtained from slaughter-houses in and around Tirupati, necropsied animals at the Department of Veterinary Pathology, College of Veterinary Science, Tirupati, and field mortality cases.

Table 1. Occurrence (%) of neoplastic conditions in the sheep ovaries.

S. No.	Name of the neoplastic condition	Number of cases	Occurrence (%)
	Sex cord neoplasm		
1.	Granulosa cell tumour	16	41.03
	Epithelial neoplasms		
2.	Cystadenoma	10	25.64
3.	Adenocarcinoma	2	5.12
4.	Clear cell carcinoma	2	5.12
	Mesenchymal neoplasms		
5.	Hemangiosarcoma	4	10.25
6.	Fibroma	2	5.12
7.	Angioleiomyoma	2	5.12
8.	Hemangiopericytoma	1	2.56
	Total	**39**	**100**

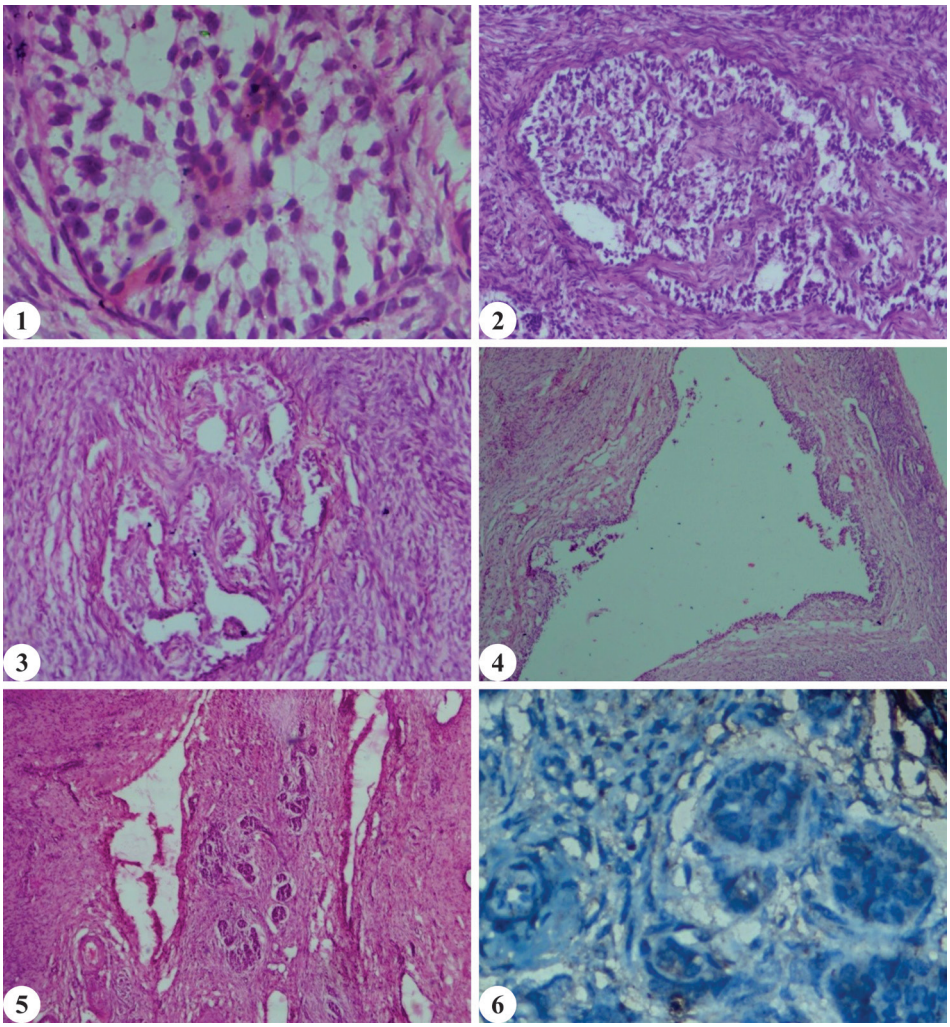

Fig. 1. Granulosa cell tumour: Neoplastic granulosa cells forming Call-Exner bodies, arranged in a distinctive rosette pattern around eosinophilic material (H&E x400); **Fig. 2.** Multifocal coalesced granulosa cell tumour: The infiltrated fibrous tissue made the neoplastic area into a multifocal coalesced pattern (H&E x100); **Fig. 3.** Cystadenoma of the ovarian stroma: Neoplastic epithelial cells projecting into the cystic space are supported by collagenous fibers (H&E x100); **Fig. 4.** Cystadenoma of rete ovarii: Irregular cystic space near the hilus surrounded by a thick layer of proliferated fibrous tissue, with the lining epithelium desquamated into the lumen (H&E x400); **Fig. 5.** Adenocarcinoma: Neoplastic epithelial cells arranged in a tubulo-acinar pattern in the ovarian stroma and supported by fibrous stroma (H&E x40); **Fig. 6.** Adenocarcinoma: Pan cytokeratin revealed mild cytoplasmic positivity within the neoplastic cells, observed as a brown color (IHC x400).

Necropsies were performed according to standard protocols[7]. For histological analysis, the ovaries were first subjected to a gross examination and then fixed in 10% neutral buffered formalin. The tissues were processed through a graded alcohol series, cleared with xylene, embedded in paraffin and cut into 4-5 µm thick sections using a Leica manual rotary microtome. These sections were then deparaffinized, rehydrated through decreasing alcohol concentrations and stained with hematoxylin and eosin (H&E) following standard procedures[8].

For IHC duplicate paraffin sections of the ovary were cut at 3-4 µm thickness, mounted on 3-Aminopropyltriethoxysilane (APES) coated slides, and incubated overnight at 37^{O}C. Sections were deparaffinized in xylene (two changes, 15 minutes each), rehydrated through graded alcohols and washed in distilled water. Antigen retrieval was performed using the heat-induced method with a pressure cooker (three whistles) in Tris-EDTA buffer (pH 9.0) and endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 minutes. Sections were washed in Tris buffer (pH 7.6) and incubated with primary antibodies (VEGF, Pan Cytokeratin and SMA) for 30 minutes at room temperature (25±5). After washing in Tris-EDTA buffer, super enhancer solution was applied for 20 minutes, followed by a secondary antibody with poly-horseradish peroxidase (Poly-HRP) for 20 minutes. Diaminobenzidine (DAB) was used as the chromogen and sections were counterstained with Harris hematoxylin, blued in lithium carbonate and mounted in DPX (kits from Bio Genex, USA).

RESULTS

The study identified eight ovarian neoplasms: one sex cord-stromal neoplasm (granulosa cell tumoUr), three epithelial neoplasms (adenoma, adenocarcinoma and clear cell carcinoma) and four mesenchymal neoplasms (hemangiosarcoma, fibroma, angioleiomyoma and hemangiopericytoma) (Table 1). Granulosa cell tumour was the most prevalent, followed by adenoma and hemangiosarcoma. While no gross lesions were characteristic, histopathological and immunohistochemical evaluations were instrumental in tumor classification and characterization.

Granulosa cell tumours were observed in two forms: (1) the predominant form, characterized by intrafollicular radial aggregation of tumour cells around eosinophilic material (Call-Exner bodies) (Fig. 1) and (2) the multifocal coalesced pattern, where lobules of granulosa cells were separated by fibrous tissue. Neoplastic cells in both forms exhibited hyperchromatic nuclei with minimal cytoplasm (Fig. 2).

Two types of cystadenomas were identified: cystadenoma of the ovarian stroma and cystadenoma of the rete ovarii. The former featured neoplastic epithelial cells that were polyhedral in shape, well-oriented, and had round to ovoid nuclei with little cytoplasm and few mitotic

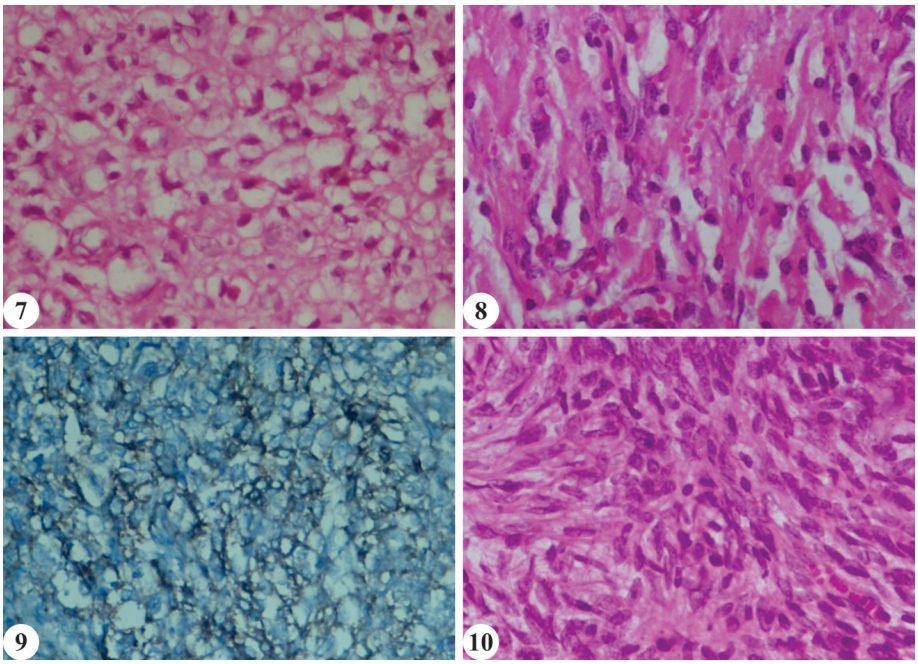

Fig. 7. Clear cell carcinoma: Clear cells with empty cytoplasm and peripherally placed nucleus having a signet ring appearance (H&E x400); **Fig. 8.** Hemangiosarcoma: Proliferated endothelial cells forming clefts supported by thin collagen fibers. The cleft contained few RBCs some without RBCs (H&E x400); **Fig. 9.** Hemangiosarcoma: Cytoplasm of neoplastic endothelial cells showing moderate immunopositivity with VEGF (IHC x400); **Fig. 10.** Fibroma: Spindle shaped fibroblasts with oval nuclei, prominent nuclear borders and dispersed chromatin with multiple nucleoli (H&E x400).

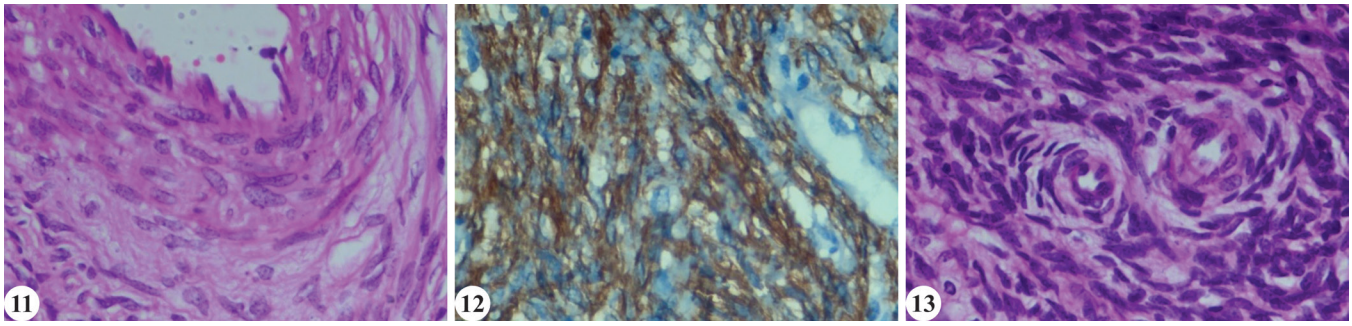

Fig. 11. Angioleiomyoma: Neoplastic smooth muscle cells are proliferating towards the ovarian stroma from the blood vessels (H&E x400); **Fig. 12.** Angioleiomyoma: Immunoexpression of SMA gives a brown colour to the reactive neoplastic cell's cytoplasm and cell outlines (IHC x400); **Fig. 13.** Hemangiopericytoma: Proliferated neoplastic pericytes giving fingerprint like appearance (H&E x400).

figures. Collagen fibers projected into the cystic lumen, with the cystic wall lined by thick collagenous fibers (Fig. 3). The latter, located near the hilus, was characterized by an irregular shape, lined by low cuboidal epithelial cells and enclosed by a fibrous capsule surrounding the cystic space. The neoplastic cells within the cystic space were round to oval in shape, with little cytoplasm and round to elongate nuclei and exhibited few mitotic figures (Fig. 4).

Adenocarcinoma was characterized by neoplastic cells arranged in a tubulo-acinar pattern within a thin supportive connective tissue stroma. The pleomorphic neoplastic cells exhibited round to oval nuclei with scant cytoplasm (Fig. 5). Pan-cytokeratin immunostaining showed mild cytoplasmic positivity in the neoplastic cells, observed as a brown coloration (Fig. 6).

Clear cell carcinoma was identified by cells with clear cytoplasm and peripherally displaced oval to flattened nuclei, giving a typical signet ring appearance (Fig. 7).

Hemangiosarcoma showed as multifocal areas of proliferated and thickened blood vessels with undifferentiated endothelial cells infiltrating the ovarian stroma. The proliferating endothelial cells formed clefts supported by thin collagen fibers. These clefts contained a few red blood cells (RBCs), while some were devoid of RBCs. The proliferating endothelial cells were spherical, ovoid or polygonal in shape, with scant cytoplasm and tapering cytoplasmic processes (Fig. 8). IHC revealed moderate VEGF biomarker immunopositivity in the proliferating endothelial cells (Fig. 9).

Fibroma consisted of whorls and interlacing bundles of mature fibroblasts and collagen within the ovarian stroma. The neoplastic fibrocytes were uniform sized and fusiform in shape, with large nuclei that were normochromatic to hyperchromatic and oval to elongated in shape, containing multiple nucleoli (Fig. 10).

Angioleiomyoma exhibited interlacing bundles of strap-like smooth muscle fibers arranged in an intersecting pattern. The spindle-shaped neoplastic cells had cigar-shaped nuclei with rounded, blunt ends (Fig. 11). Immunostaining for smooth muscle actin (SMA) demonstrated diffuse moderate-to-strong cytoplasmic positivity in myoepithelial cells, smooth muscle cells, myofibroblasts and neoplastic cells (Fig. 12).

Hemangiopericytoma was characterized by neoplastic cells proliferating around blood vessels in a whorl pattern, creating a characteristic fingerprint appearance. The spindle-shaped neoplastic cells had short to moderate cytoplasmic processes. Their nuclei were ovoid to elongated, single and prominent, with some exhibiting chromatin margination along the nuclear membrane (Fig. 13).

DISCUSSION

Ovarian tumours are a common finding in many domestic animals, particularly in bitches and cows[3]. However, due to the practice of culling infertile sheep, the occurrence and characteristics of ovarian neoplasms in this species have not been extensively documented. This study aimed to bridge this knowledge gap by investigating the occurrence and characterizations of different types of ovarian neoplasms in sheep. The findings revealed a significant occurrence of ovarian neoplasms in sheep, comprising one sex cord-stromal neoplasm, three epithelial neoplasms and four mesenchymal neoplasms at the microscopic level. However, none of the ovaries exhibited distinct gross neoplastic changes, which may be attributed to the early culling of the sheep, preventing sufficient time for the development of gross lesions.

The most common neoplastic condition observed was the granulosa cell tumour (GCT), which presented with two distinct histomorphological patterns. The first was the classic form, characterized by radial aggregates of tumor cells surrounding a central deposit of eosinophilic proteinaceous material, known as Call-Exner bodies. This is considered the typical manifestation of GCT[4,5]. The second pattern was multifocal and coalescing, likely representing a combined form of different histological patterns of GCT[9]. In cases of cystadenoma, including cystadenoma of the ovarian stroma and cystadenoma

of the rete ovarii, the neoplastic cells were observed as long papillary fronds or short digitating projections, with some exhibiting a pseudoglandular pattern[3]. The histologic appearance of adenocarcinoma, characterized by proliferating neoplastic cells arranged in a tubulo-acinar pattern with a thin supportive connective tissue stroma, is a consistent finding[10]. The microscopic characterization of the Clear cell carcinoma as polyhedral cells with clear to eosinophilic cytoplasm, peripherally displaced oval to flattened nuclei and diffuse sheets of cells separated by collagenous septa is aligned with the findings of Tavassoli and Devilee[11]. The histological features of Hemangiosarcoma described by Jangir *et al.* are consistent with those found in the current study, specifically being characterized by the presence of clefts formed by undifferentiated endothelial cells. These clefts were supported by thin collagen fibers and sometimes contained red blood cell[12]. According to Moulton, ovarian fibroma is characterized by whorls and interlacing bundles of mature fibroblasts and collagen within the ovarian stroma, a feature corroborated by other authors[5,13]. The same microscopic features of ovarian fibroma were also evident in this study. The microscopic pattern of angioleiomyoma in this study exhibited interlacing bundles of strap-like smooth muscle fibers arranged in an intersecting pattern, as reported previously[14]. The histologic characterization of angioleiomyoma as interlacing bundles of strap-like smooth muscle fibers arranged in an intersecting pattern was reported earlier with similar features[14]. Hemangiopericytoma, observed in this study, appeared as neoplastic cells proliferating around blood vessels in a whorl pattern, creating a fingerprint-like appearance, similar features to those reported in other studies[4,15].

To further characterize these tumours, we performed IHC using specific biomarkers. Hemangiosarcoma showed moderate immunopositivity for VEGF within the proliferating endothelial cells, a finding consistent with previous reports in canine splenic hemangiosarcoma[16]. Sections histologically identified as adenocarcinoma exhibited mild cytoplasmic positivity for pan-cytokeratin within the neoplastic cells, which aligns with the earlier findings on epithelial tumors[17]. Smooth muscle actin demonstrated diffuse moderate-to-strong positivity in myoepithelial cells, smooth muscle cells, myofibroblasts and neoplastic cells in angioleiomyoma, which is consistent with previous studies[18].

This study highlights the significant occurrence and diverse histopathological patterns of ovarian neoplasms in sheep. Our findings, supported by specific immunohistochemical markers such as VEGF, pan-cytokeratin and SMA, provide valuable insights into the characteristics and differentiation of sex cord-stromal, epithelial and mesenchymal ovarian tumours in this species. The results are largely consistent with established literature, emphasizing the importance of detailed pathological examination in diagnosing these conditions in sheep and contributing to the limited body of knowledge on this subject.

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