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Case Report

Ovarian granulosa cell tumor in bovine fetus

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Abstract
Ovarian granulosa cell tumor (GCT) is a common type of sex cord neoplasm in cattle. This paper reports a rare case of GCT identified in a bovine abortion. A necropsy was performed on an 8-month-old bovine fetus, revealing an enlarged right ovary measuring 5.5 cm. The cut surface displayed reddish cystic areas with mucinous content, interspersed with whitish to blackish regions. The histopathological evaluation identified a benign epithelial neoplastic proliferation with an acinar arrangement supported by fibrocollagenous stroma. Immunohistochemistry was performed using antibodies against cytokeratin AE1/AE3, vimentin, protein S-100, chromogranin A, inhibin, and somatostatin. The tumor stained positive for cytokeratin AE1/AE3 and vimentin, while protein S-100, inhibin, chromogranin A, and somatostatin were non-reactive. Polymerase chain reaction (PCR) tests for *Toxoplasma gondii*, *Neospora caninum*, and bovine viral diarrhea virus yielded negative results. This report provides a detailed anatomical, histopathological, and immunohistochemical characterization of a congenital GCT in a bovine fetus.

Keywords: abortion, congenital tumor, fetus, neoplasia.

Introduction

Primary ovarian neoplasms can be classified into tumors of the surface germinal epithelium (e.g., papillary adenomas, papillary adenocarcinomas, cystadenomas, and undifferentiated carcinomas), tumors of the sex cord-stromal tissues (e.g., granulosa cell tumors, thecomas, and luteomas), and tumors originating from germ cells (e.g., dysgerminomas and teratomas) (6, 22).

Ovarian granulosa cell tumor (GCT) is a neoplasm arising from the sex cord stroma of the ovaries and represents the most common type of ovarian tumor in cattle (3, 25). The occurrence of GCT tends to increase with age, being more prevalent in adult animals, and primarily affects equine (8, 26), bovine (14, 20), and human species (9). Typically, GCT presents as a unilateral, benign tumor (18). This neoplasm can affect all bovine breeds but is more frequently diagnosed in dairy cattle than beef cattle, likely due to the more intensive reproductive monitoring in dairy breeds (21).

The most common clinical signs of GCT include hormonal imbalances caused by increased steroid production. Consequently, affected females may exhibit estrus during anestrus, continuous or intermittent estrus, or even masculine behavior (25). Neoplastic granulosa cells inhibit the release of follicle-stimulating hormone (FSH) through inhibin synthesis, which, in turn, leads to contralateral ovarian atrophy (6). Additionally, tumor rupture may result in abdominal pain and hemoperitoneum (23).

The objective of this study is to report a case of GCT in the ovary of a bovine fetus and provide a detailed description of its anatomopathological and immunohistochemical findings.

Case description

A necropsy was performed on a female crossbred bovine fetus at a gestational age of 8 months. The farm

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primarily focuses on dairy production, with cattle kept on native pasture supplemented with additional feed and mineral salt. During the necropsy, the right ovary was notably enlarged, measuring 5.5 cm in diameter. Upon sectioning, the ovary exhibited diffuse reddish discoloration, with multifocal to coalescent areas ranging from black to whitish tones, as well as multiple cystic formations measuring 0.2–0.5 cm in diameter containing mucinous material (Fig. 1). The left ovary showed no abnormalities. Samples from the enlarged ovary were collected and submitted for histopathological analysis.

Slides containing histological sections of the ovarian neoplasm were sent to the Adolfo Lutz Institute for immunohistochemical examination. Antigen retrieval was performed using wet heat (10 mM citric acid solution, pH 6), and the sections were incubated at 4°C for 12 hours with the following antibodies: anti-cytokeratin AE1/AE3 (mouse monoclonal, Biocare Medical, CA, USA; dilution 1:2000), anti-vimentin (mouse monoclonal [V9], Invitrogen/Thermo Fisher Scientific, CA, USA; dilution 1:800), anti-protein S-100 (rabbit polyclonal, Dako/Agilent, CA, USA; dilution 1:800), anti-chromogranin A (mouse monoclonal [LK2H10+PHE5], Biocare Medical, CA, USA; dilution 1:1000), anti-alpha-inhibin (mouse monoclonal [BC/R1], Biocare Medical, CA, USA; dilution 1:100), and anti-somatostatin (rabbit polyclonal, Dako/Agilent, CA, USA; dilution 1:100). Detection and revelation were conducted using secondary antibodies conjugated to peroxidase and diaminobenzidine chromogen (Reveal Detection System Polyvalent HRP DAB, Spring Bioscience, CA, USA). Positive and negative controls were processed alongside the test samples.

Polymerase chain reaction (PCR) was performed on frozen brain tissue to detect *Neospora caninum* and *Toxoplasma gondii*. DNA extraction was conducted using the phenol-chloroform method (12). For *T. gondii* detection, the primers SAG2.F4 (5'-GCTACCTCGAACAGGAACAC-3') and SAG2.R4 (5'-GCATCAACAGTCTTCGTTGC-3') were used to amplify a 332 bp product of the SAG2 gene (31). For *N. caninum*, the primers Np21 (5'-CCCAGTGCGTCCAATCCTGTA-3') and Np6 (5'-CTCGCCAGTCAACCTACGTCTTCT-3') were used to amplify a 337 bp product from the Nc5 region (7). Positive controls included purified tachyzoites of *T. gondii* and *N. caninum* (Nc-Bahia strain), while negative controls consisted of autoclaved ultrapure water.

Reverse transcription-polymerase chain reaction (RT-PCR) was also performed on thymus and spleen samples to detect bovine viral diarrhea virus (BVDV). The primers 324F (5'-ATGCCCTTAGTAGGACTAGCA-3') and 326R (5'-TCAACTCCATGTGCCATGTAC-3') (19) were used to amplify a 288 bp product from the 5'UTR region of the pestivirus genome. All molecular analyses for the detection of infectious agents returned negative results.

Histopathological evaluation revealed a benign neoplastic proliferation arising from the sex cord stroma, expanding and replacing the right ovarian parenchyma. The lesion was non-delimited and non-encapsulated, organized in acini and ducts, and supported by moderate fibrocollagenous stroma (Fig. 2-A). The neoplastic cells were polyhedral, with centrally located round to oval nuclei, loose chromatin, and prominent nucleoli. The cytoplasm was moderate, eosinophilic, and moderately distinct (Fig. 2-B). Among the neoplastic cells, multiple cystic dilations and areas of moderately extensive focal hemorrhage were evident. Moderate anisocytosis and anisokaryosis were also observed.

Immunohistochemical analysis showed positive immunostaining for cytokeratin AE1/AE3 (Fig. 2-C) and vimentin (Fig. 2-D). Staining for chromogranin A, inhibin, and somatostatin was non-reactive, while protein S-100 was negative. These histopathological and immunohistochemical findings were consistent with a granulosa cell tumor.

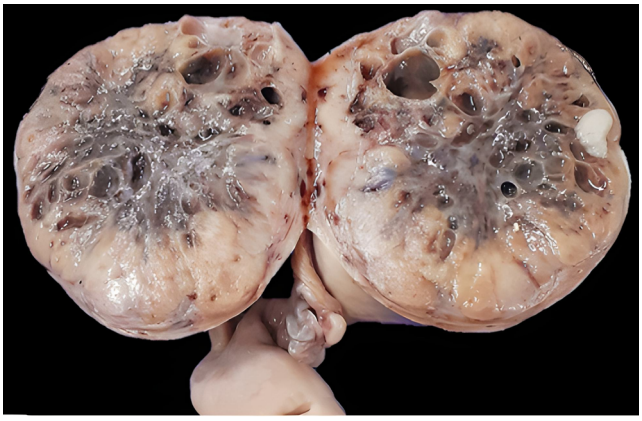

Figure 1. Ovarian granulosa cell tumor in a bovine fetus. The right ovary was enlarged, measuring 5.5 cm in diameter, with whitish to blackened areas upon sectioning and multiple cysts ranging from 0.2 to 0.5 cm in diameter.

Discussion

Congenital tumors are defined as neoplasms detected during gestation or within the first two months after birth (2). These tumors have varied etiologies and represent a limited subset of diagnoses across all species, including cattle (13). In this report, we established the diagnosis of a granulosa cell tumor (GCT) in a bovine fetus through anatomopathological findings and immunohistochemical characterization. GCTs are typically reported in adult animals, often as incidental findings during necropsy (29).

In heifer calves, various types of reproductive system neoplasms have been documented, including embryonal carcinoma (1), mesothelioma (24), Sertoli cell tumor (30), and teratomas (11). Regarding GCTs, only one prior case has been reported in a bovine neonate, where an enlarged left ovary with a lobulated surface and multiple cavitations filled with reddish-brown serous fluid was described. In contrast, the contralateral ovary was reduced in size (17). In our case,

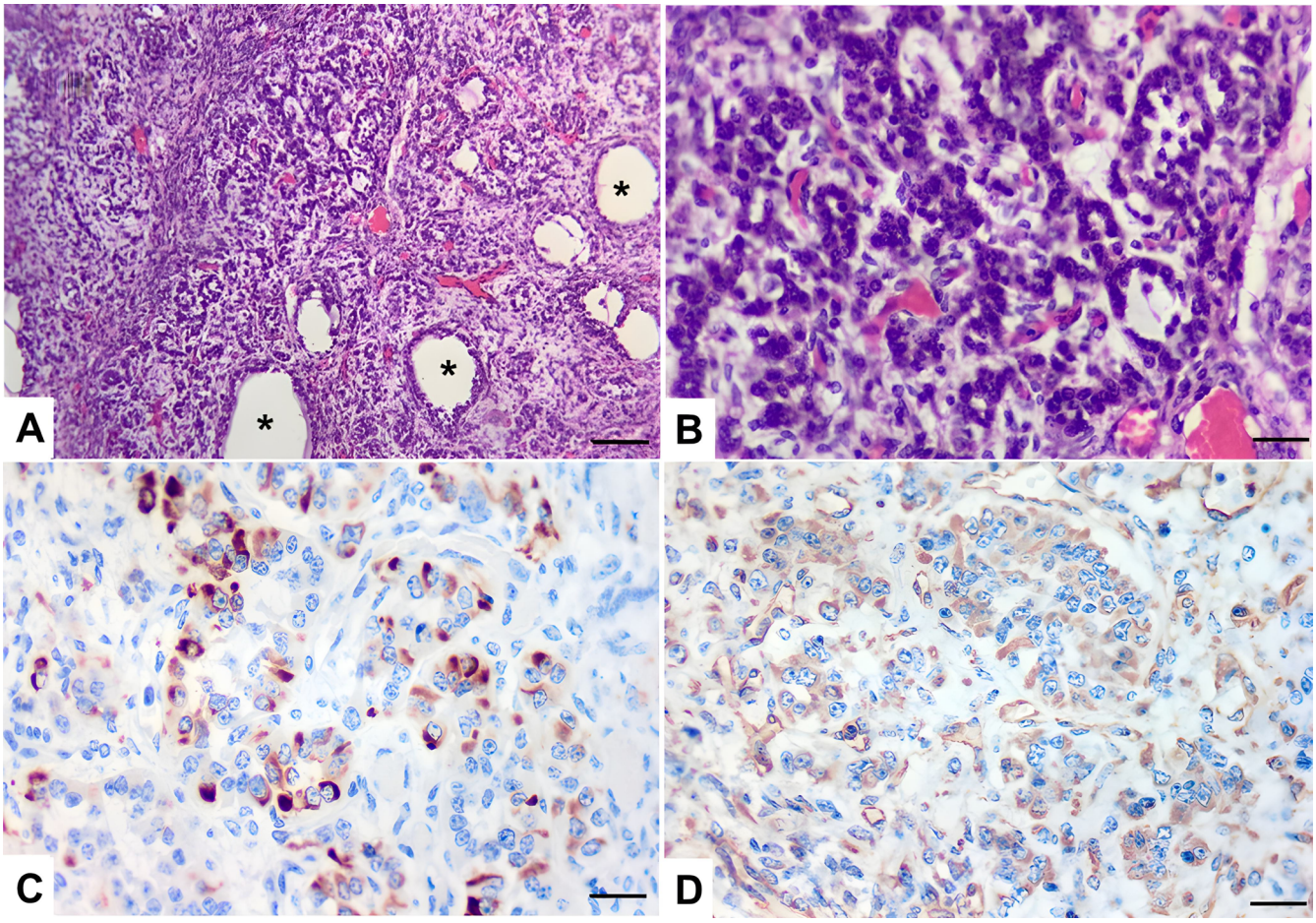

Figure 2. Histopathological and immunohistochemical patterns of an ovarian granulosa cell tumor in a bovine fetus. (A) Benign neoplastic proliferation of sex cords arranged in acinar and ductal patterns, with multiple cystic formations (*) supported by moderate fibrocollagenous stroma. Hematoxylin and eosin (HE), Obj. x10. Scale bar: 100 µm. (B) Neoplastic polyhedral cells are organized in ducts with rounded, centrally located nuclei and moderate, eosinophilic, and moderately distinct cytoplasm. HE, Obj. x40. Scale bar: 20 µm. (C) Moderate positive immunostaining in the cytoplasm of neoplastic cells for cytokeratin AE1/AE3. Immunohistochemistry (IHC), Obj. x40. (Anti-cytokeratin AE1/AE3: polymer linked to endogenous peroxidase, chromogen diaminobenzidine [Reveal Detection System Polyvalent HRP DAB, Spring Bioscience, CA, USA]). (D) Moderate positive immunostaining in the cytoplasm of neoplastic cells for vimentin. IHC, Obj. x40. (Anti-vimentin: polymer linked to endogenous peroxidase, chromogen diaminobenzidine [Reveal Detection System Polyvalent HRP DAB, Spring Bioscience, CA, USA]).

the right ovary was enlarged, and the left ovary showed no macroscopic alterations.

Macroscopic characteristics of GCTs in adult animals and other species often include moderate to significant unilateral ovarian enlargement with an irregular or multilobulated surface. On sectioning, a cystic pattern with serous fluid interspersed with solid areas ranging from white to yellowish is commonly observed (3, 8, 14, 20, 27). Although these descriptions are based on adult animals, they align with our findings in an 8-month-old bovine fetus. Studies in cattle have reported unilateral ovarian enlargement ranging from 5 to 25 cm in diameter, with sectioning revealing multiple cavities filled with serosanguinous or serous fluid, along with solid whitish tissue of moderate consistency (5, 14, 20, 28), which corresponds to the observations in our study.

GCTs are generally benign, although malignant variants may exhibit combinations of microfollicular, macrofollicular, acinar, tubular, trabecular, and solid cellular arrangements (3, 8, 15). A study described a malignant GCT characterized by solid and tubular cellular arrangements, marked anisocytosis and anisokaryosis, numerous mitotic figures (including atypical ones) per 2.37 mm², binucleated cells, and multifocal necrosis (20). In our study, the observed cellular pattern was acinar, and no criteria for malignancy were identified, supporting the diagnosis of a benign GCT.

Immunohistochemical analysis is fundamental in confirming tumors like GCTs. Typical immunohistochemical

markers for GCTs include cytokeratin, vimentin, inhibin, desmin, and glutathione S-transferase (10, 28). GCTs are characterized by the co-expression of cytokeratin, vimentin, and inhibin in tumor cells, in addition to the relevant histopathological features of the neoplasm (8). However, in our study, inhibin, chromogranin A, and somatostatin markers did not show cross-reactivity with bovine tissues, as these antibodies were designed for human tissues. A study investigating immunohistochemistry for FIV in cats using human antibodies demonstrated that 67 out of 95 antibodies tested showed no reactivity with feline tissues (16), supporting the lack of cross-reactivity observed in our study.

Tumors with co-expression of vimentin and cytokeratin are generally considered benign, with cytokeratin primarily expressed in degenerated cells (8), consistent with our findings. Negative staining for protein S-100 has been associated with poorly differentiated GCTs (10), which aligns with our results, as the tumor cells in this case were poorly differentiated.

This case represents one of the few descriptions of congenital GTC in a bovine fetus. The histopathological pattern, combined with immunohistochemical results showing positive staining for cytokeratin AE1/AE3 and vimentin, is consistent with a poorly differentiated benign tumor.

Conflict of Interest

The authors declare no competing interests.

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