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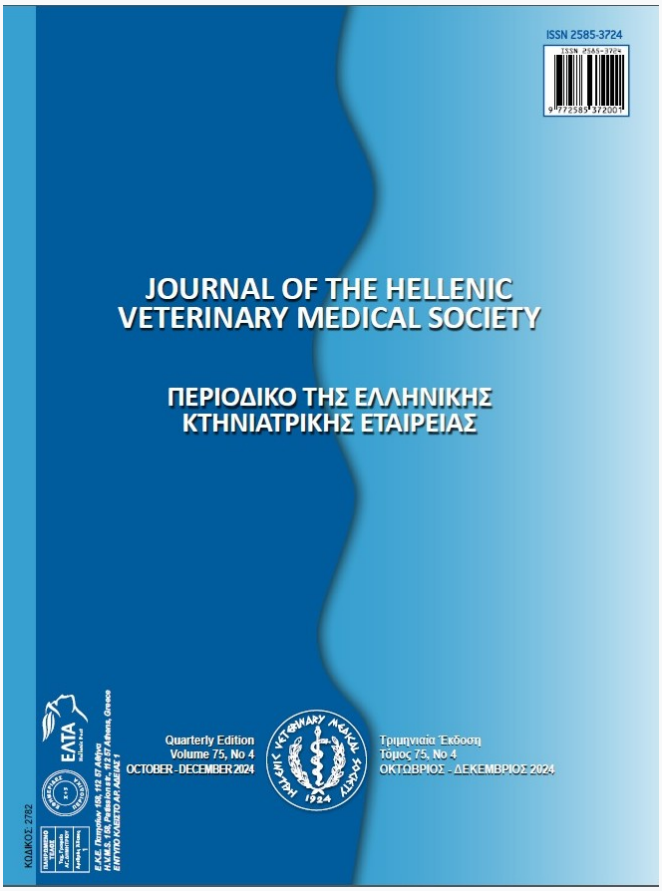

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Case report
Κλινικό περιστατικό

Myocardial Epithelial Inclusions in a Bovine Fetus

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ABSTRACT. Myocardial epithelial inclusions (MEIs) were identified in a 6-month-old aborted Simmental breed fetus through gross, histopathological, and immunohistochemical assessments. Grossly; a circular, unencapsulated white lesion, in 2.5 mm diameter was detected on the free wall of the left ventricular myocardium. Histopathological examination showed a densely cellular focus with tubular, ductular, or acinar structures surrounded by abundant collagen. Immunohistochemically, the acinar and tubular cells showed strong cytoplasmic immunoreactivity to cytokeratin and vimentin. As a result, this report represents first reported case MEI in a bovine fetus and it emphasizes the necessity of add the differentials of MEIs during postmortem examinations, including the fetal period in cattle.

Key words: *Bovine; Epithelial Inclusions; Fetus; Immunhohisochemistry Myocardium; Pathology.*

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INTRODUCTION

Myocardial Epithelial Inclusions (MEIs) have been reported in various domestic animal species (Robinson and Robinson 2016), with a higher frequency observed in calves and adult cattle (Guarda et al.; 2004, .Baker et al.; 1992, Nordstoga and Aleksandersen 1988, Tursi et al.; 2009, Jolly 1965). However, there are no reports of MEIs occurring during fetal development in cattle. Nevertheless, it has been documented during embryonic development in human (Aqel and Sousha 1994), the case presented here is the first reported instance of myocardial inclusion in veterinary species, where such findings have not been documented previously in fetal period. Clinically, myocardial epithelial inclusions (MEIs) in cattle do not typically induce cardiac signs (Baker et al.; 1992, Nordstoga and Aleksandersen 1988). However, in humans, cases of epithelial inclusions in the atrial node and interatrial septum reportedly caused heart block (Barr and Pollock 1968). Therefore, it is important during postmortem examinations to accurately identify MEIs to prevent misinterpretation of this structure. In this report, we present findings of gross, histological, and immunohistochemical findings of myocardial epithelial inclusions detected in a 6-month-old aborted fetus of the Simmental breed.

CASE HISTORY

A 6-month-old bovine Simmental fetus was submitted to the Department of Veterinary Pathology for pathological examination. After autopsy, tissue samples were collected from myocardium, lung, brain, kidneys, liver for histopathologic, virologic and microbiologic examinations. Virologically; Bovine Viral Diarrhea Virus, Border Disease Virus and Bovine Herpesvirus-1 were tested by PCR using proper primers. Microbiologically, aerobic and anaeorobic cultures were used for microbiological cultivation. Tissue samples were fixed in 10% formalin and subsequently embedded in paraffin. Histological sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome (MTC). Additionally, immunohistochemical staining was performed using the avidin-biotin-peroxidase complex method for cytokeratin (Dako, 1/100) and vimentin (Dako, 1/100) antibodies by using autostainer (Ventana NexES system). The microslides were examined using an Olympus BX42 microscope equipped with a DP72 camera (Olympus cellSens).

RESULTS

Upon gross examination; a circular, unencapsulated, single, white lesion, with a diameter of up to 2.5 mm, was identified on the free wall of the left ventricular myocardium (Fig-1A). No significant lesions were detected in the remaining organs examined. Despite thorough bacteriological and virological examinations, no etiologic agent was determined.

Histological examination showed an uncapsulated, densely cellular focus characterized by tubular, ductular, or acinar structures exhibiting a single or double layer of cuboidal epithelium (Fig1-B). These structures appeared either empty or contained lumina filled with protein, occasionally forming scattered nests of cells. Abundant collagen was present surrounding and separating these structures (Fig-1C), alongside scant eosinophilic cytoplasm, round to oval nuclei devoid of mitoses or atypia. Small clusters of similar epithelial cells embedded in collagenous connective tissue were present among the tubules. Normal myocardial cells surrounded this structure. .The acinar and tubular cells showed strong cytoplasmic immunoreactivity to cytokeratin and vimentin (Fig-1D, E).

DISCUSSION

Bovine MEI'S are rare congenital changes characterized by non-proliferative, lack of metastatic potential, and absence of malignant transformation (Robinson and Robinson 2016, Baker et al.; 1992, Nordstoga and Aleksandersen; 1988, Tursi et al.; 2009, Jolly 1965). Consistent with the present case, these structures predominantly occur in the left ventricular free wall near the apex of the heart (Baker et al.; 1992, Nordstoga and Aleksandersen; 1988, Tursi et al.; 2009, Jolly 1965).

Microscopically, MEI consisted of squamous to cuboidal epithelial cells, forming various structures such as tubules, ducts, and acini. Similar cellular compositions and structures have been documented in prior reports (Guarda et al.; 2004, Kaspareit et al.; 2003, Thomas and Van Wesep 1990, Tursi et al.; 2009, Baker et al.; 1993)

The detection of MEI in a six-month-old prenatal calf in this study indicates the possibility of early formation of these inclusions during embryonic development.. This finding may support the idea that these inclusions arise during the early stages of cardiac embryogenesis (Nordstoga, Aleksandersen, 1988; Jolly, 1965). However, the precise etiology of MI's in cattle

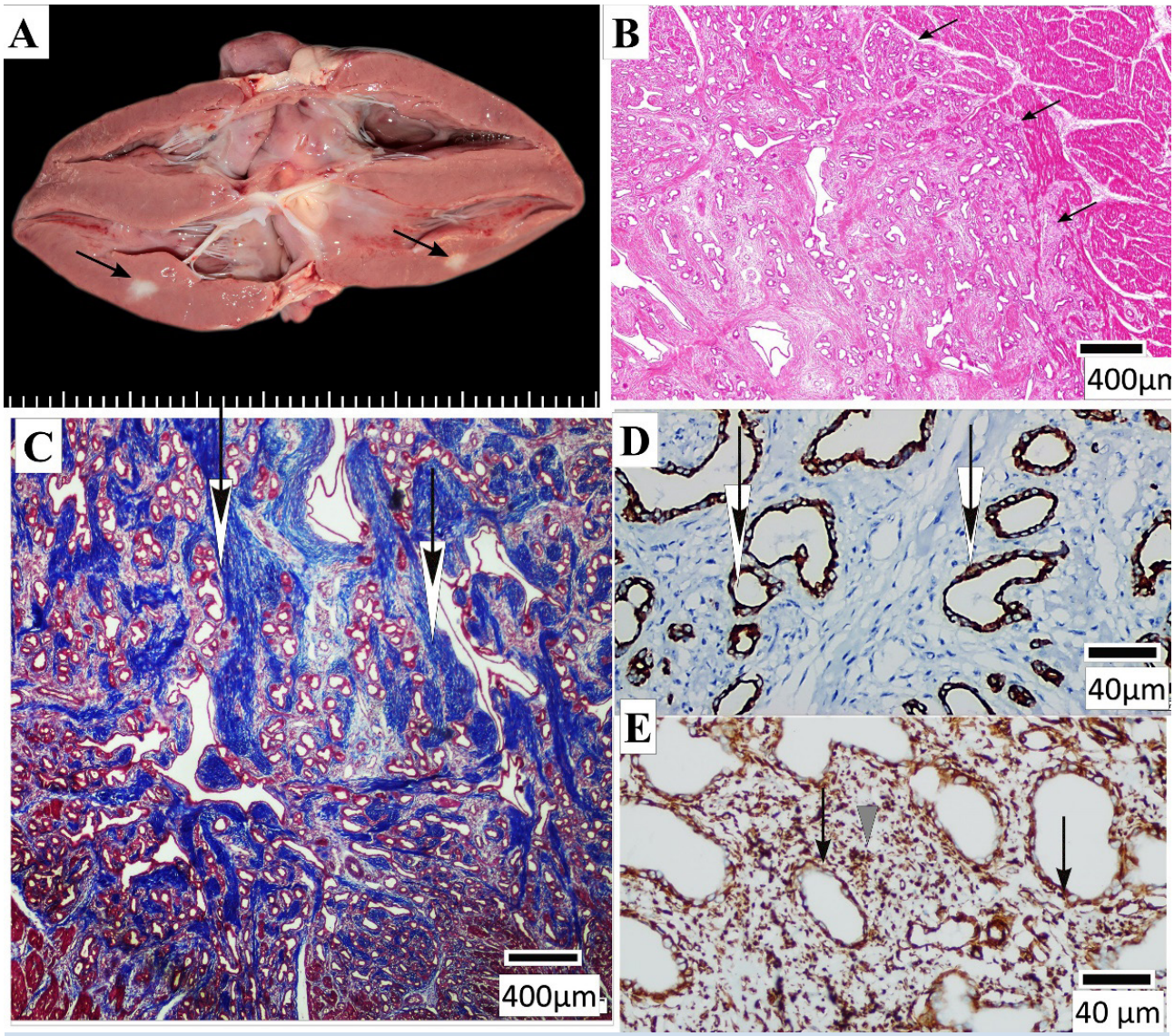

Figure 1-A: Gross appearance of cut surfaces heart containing white, round focus (arrow) measuring 2-3 mm diameter.
Figure 1-B: Histopathological appearance of inclusion cyst containing multiply distributed acini (arrows) covered by normal cardiac muscle cells, H-E, x4.
Fig.1-C: Fibrous tissue distribution amongst the acinar structures, x4, Masson trichrome
Fig.1-D: Tubular and acinar cells exhibit multifocal expression of cytokeratin (arrows Immunoperoxidase reaction with DAB chromogen and counterstained with Harris hematoxylin, x40.
Fig.1-E: Acinar cells (arrow) and interstitial fibroblasts (arrow head) show positive vimentin expression. Immunoperoxidase reaction with DAB chromogen and counterstained with Harris hematoxylin, x40.

remains incompletely understood. One current theory suggests that they are choristomas, originating when foregut epithelium becomes entrapped within the dorsal mesocardium prior to the closure of the pleuropericardial fold (Baker et al.; 1993). An alternative hypothesis is a potential derivation from endodermal (Jolly 1965, Guarda et al.; 2004) or mesothelial origins (Tursi et al.; 2009). Cytokeratin and vimentin are intermediate filaments within the animal body. Vimentin is the most prevalent intermediate filament present throughout body of animals. tissues. Embryologically, vimentin is the initial filament expressed, and plays a key role in cellular structure. However, as development progresses, it undergoes replacement by filaments specific to individual cell types except for mesenchymal cells (Strouhalova et. al.,2020). The co-expression of vimentin and cytokeratin is a characteristic shared by certain malignant tumors, cultured epithelial cells, as well as mesothelial, granulosa, and rete ovarii cells within the human ovary (Strouhalova

et. al.,2020, Viale G ., et. al.1988). Previously documented findings indicated that the cells of MEIs consistently exhibit positive immunostaining for cytokeratin, with the potential to exhibit either positive or negative immunoreactivity to vimentin (Tursi et al.; 2009, Baker et al.; 1993). The positive expression of both markers in this present study could imply their origin from displaced foregut epithelium, which might have been sequestered in the dorsal mesocardium prior to the closure of the pleuropericardial folds during early embryonic development (Kaspareit et al.; 2003, Thomas and Van Wesep 1990). However, endothelial origin can not be totally excluded.

In the present report, positive immunoreactivity of acinar cells to vimentin and cytokeratin might suggest mesothelioma, despite lacking histological features typical of it. For these reasons, MEIs may be mistakenly diagnosed as metastatic adenocarcinoma or intracardiac mesothelioma..The findings from ultrastructural examination, including elongated microvilli and well-formed desmosomes linked with tonofibrils (Tursi et al.; 2009), provide further evidence in favor of the cells' origin from the foregut.

In comparative pathology, similar epithelial structures have been reported as squamous cysts and plaques in the myocardium of cynomolgus monkeys (Kaspareit et al.; 2003) and humans (Aqel and Sousha 1994, Barr and Pollock 1968, Thomas and Van Wesep 1990, Hopkinson and Newcombe 1971). However, all these examples contained cystic structures. In this report, there were no cystic or squamous changes, contrary to what is typically reported in humans (Barr and Pollock 1968, Thomas and Van Wesep 1990, Hopkinson and Newcombe 1971).

As a result, this report represents first case of MEI in a bovine fetus and it emphasizes the necessity of add the differentials of MEIs during postmortem examinations, including the fetal period in cattle.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

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