



Clinical, sonographic and anatomopathological aspects of ovarian teratoma in a heifer

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ABSTRACT: *Teratomas rarely occur in domestic species, especially in cattle. These tumors originate in fetal life and are characterized by rapid growth, which justifies their frequent detection in young animals. This study reported a case of ovarian teratoma in a heifer. On physical examination, the main signs identified were apathy, abdominal distention and tension, empty rumen, and mushy diarrhea. During rectal palpation, a mass was identified in the pelvic region, which was suggestive of cysts on ultrasound examination. The animal underwent laparotomy, followed by euthanasia due to a poor prognosis. At necropsy, a 54 x 43 x 52 cm (length x width x thickness) tumor was observed in the right ovary with multiple cystic areas, in addition to masses associated with multiple adhesions of the intestinal loops and peritonitis. On histopathology, muscle, cartilage, bone, nervous and epithelial tissue, glands, hair with follicles, were identified in the affected ovary. There was mixed inflammation and foci of necrosis observed with a complete absence of ovarian architecture in both the ovaries. Infiltrations were identified in the lymph nodes and mesenteric vessels. Glandular ducts were seen from the serosa to the intestinal mucosa. A locally infiltrative and expansile ovarian teratoma was diagnosed accordingly. It is considered that this kind of tumor can induce abdominal distension and absence of estrus in previously healthy, non-pregnant heifers.*

Key words: *cattle, reproductive system, neoplasm, tumor.*

Aspectos clínicos, ultrassonográficos e anatomopatológicos do teratoma ovariano em uma novilha

RESUMO: *Os teratomas são tumores raros nas espécies domésticas, sobretudo em bovinos. Esses tumores são caracterizados por crescimento rápido e origem durante a vida fetal, o que justifica seu diagnóstico frequente em animais jovens. O presente trabalho relata um caso de teratoma ovariano em uma novilha. No exame físico, os principais sinais identificados foram apatia, distensão e tensão abdominal, rúmen vazio e diarréia pastosa. Durante a palpação retal, uma massa foi identificada na região pélvica. Ao exame ultrassonográfico, a massa era sugestiva de cistos. O animal foi submetido a laparotomia, seguido de eutanásia devido ao mau prognóstico. Na necropsia foi observado um tumor de 54 x 43 x 52 cm (comprimento x largura x espessura) no ovário direito com múltiplas áreas císticas, além de massas associadas à múltiplas aderências das alças intestinais e peritonite. Na histopatologia foram identificados no ovário acometido a presença de tecidos muscular, cartilaginoso, ósseo, nervoso e epitelial, com glândulas, pelos e folicúlos. Havia inflamação mista e focos de necrose com ausência completa de arquitetura ovariana em ambos os ovários. Infiltrações foram identificadas em linfonodos e vasos mesentéricos. Ductos glandulares foram vistos desde a serosa até mucosa intestinal. Diagnosticou-se um teratoma ovariano localmente infiltrativo e expansivo. Considera-se que este tumor pode induzir distensão abdominal e ausência de estro em novilhas previamente sadias e não gestantes.*

Palavras-chave: *bovinos, sistema reprodutivo, neoplasma, tumor.*

Ovarian neoplasms are classified according to the embryonic derivation of the predominant cellular component. This classification includes epithelial neoplasms (based on the ovarian surface epithelium), tumors derived from the ovarian stroma (sex cord or gonodostromal elements), those of the *rete ovarii*, and germ cell tumors (AGNEW & MACLACHLAN, 2017), which also include teratomas and dysgerminomas

(MC ENTEEN, 1990). In cattle, ovarian neoplasms are uncommon (LUCENA et al., 2011), with an incidence of less than 0.5% (MIMOUNE et al., 2017). They are usually identified upon inspection in abattoirs and among these, granulosa cell tumor prevalently affects cattle (LUCENA et al., 2011).

The occurrence of teratomas is rare in any domestic species (CARLUCCIO et al., 2017), with

a higher number of diagnoses in canines (AGNEW & MACLACHLAN, 2017). In large ruminants, this neoplasm is more uncommon, and in buffaloes, the incidence ranges from 0.16% to less than 1% (MACHADO et al., 2015; PANDE et al. 2016). To date, there is no data actually available on this tumor occurring in a cattle in Brazil. In a retrospective study conducted among the same specie in Brazil, no teratoma was noticed among the 586 tumors identified in 6.706 necropsies (LUCENA et al., 2011). The rarity of this neoplasm was also noted in the study by ALI et al. (2006), who diagnosed only two cases (1.81%) of teratomas in 110 reproductive tracts of Zebu cows evaluated in an abattoir.

Teratomas result from the neoplastic activity of pluripotent cells remaining in the embryonic notochord. They are derived usually from three germ layers (ectoderm, mesoderm, and endoderm), which results in the formation of masses comprising various tissues (MC ENTEEN, 1990; CARLUCCIO et al. 2017; ALVES et al. 2018). They are classified as teratocarcinomas (malignant teratomas), which are considered rare and immature, since they contain lesser differentiated embryonic elements, besides mature structures (AGNEW & MACLACHLAN, 2017; CARLUCCIO et al. 2017) and dermoid cysts. These are more commonly reported in Zebu cows, where they may be considered as benign or mature teratomas with clearly differentiated structures.

In the present study, we reported a case of ovarian teratoma in a nulliparous, 2-year-old, 164-kg Nelore crossbred heifer. The complaint made by the owner was that the animal had been showing progressive body mass loss and diarrhea for a year, without loss of appetite. In the past 6 months, before clinical care, the symptoms had intensified, and an increase in abdominal volume was also noted accordingly. Further, estrus or pregnancy was not reported in the animal. Physical examination identified apathy, body score 2.0 (1.0-5.0), dull bristly hair, mild dehydration (6%), mushy diarrhea, and bilateral ventral abdominal distention, most evident in the right lower quadrant (Figure 1A). On deep palpation of the right abdominal wall, the animal showed increased sensitivity. Slight tachycardia (104 beats/minute) was identified in the evaluation of vital parameters.

On rectal palpation, an abnormal structure with firm and other fluctuating areas was occupying the pelvis, upper and lower right quadrants of the abdomen, and left antimere. The mass size prevented a more detailed evaluation of the other palpable organs; however, we confirmed that the structure was

compressing the rumen, which was moderately empty. Complete blood count (CBC) and ultrasonography were requested accordingly.

CBC showed decreased values of hematocrit: 21% (24-46%), hemoglobin concentration: 72 g/dL (80-150 g/dL), mean corpuscular volume: 34.6 fL (40-60 fL), thrombocytosis $1.176 \times 10^9/L$ ($100-800 \times 10^9/L$), and platelet aggregation. Transrectal ultrasonography, performed with a 6.5-MHz linear transducer, revealed a heterogeneous echogenic structure consisting of several anechoic and hypoechoic cystic portions of varying diameters, some of them having isolated, dispersed hyperechoic points of different sizes. The cysts were delimited by hyperechoic lines, suggesting a multilocular cystic structure (Figure 1B).

The ultrasound findings indicated the need for exploratory laparotomy, which was performed in the left flank. The presence of abundant reddish fluid was identified in the abdominal cavity. During the exploration, we identified an empty rumen in the heifer. This was because there was the presence of a large mass with a firm irregular surface with some floating points, which was surrounded by some adhesions, in the right portion of the abdominal cavity. This mass had further invaded part of the left portion and had extended to the pelvis. Due to the anatomical positioning of the structure, a presumptive diagnosis of ovarian or uterine neoplasm was suggested accordingly. Owing to the large size of the tumor and infeasibility of surgical removal, the animal was submitted to necropsy after euthanasia, which was performed following intravenous administration of 10% xylazine (0.1 mg/kg), anesthesia with 10% ketamine hydrochloride (2 mg/kg), and 19.1% potassium chloride (100 mg/kg).

Significant changes were identified in the abdomen and pelvis during necropsy, mainly due to a tumor located in the right ovary (Figure 1C). The dough was round with an irregular surface, measuring 54 x 43 x 52 cm (length x width x thickness), and weighing 45 kg. On the cut surface, the tumor was multilobulated, with several cysts filled with a brownish viscous material and yellowish, solid, friable amorphous material (Figure 1D). Near the cysts, dense tissue was interpreted as bone/cartilaginous tissue (Figure 2A) with scattered circumscribed hemorrhagic spots and hair. The left polycystic ovary measured 4.0 x 3.5 x 5 cm, besides having a brownish viscous material on the cut. Both the ovaries lacked follicles and corpora lutea.

Other changes identified in the abdominal cavity included the presence of nodules, interpreted

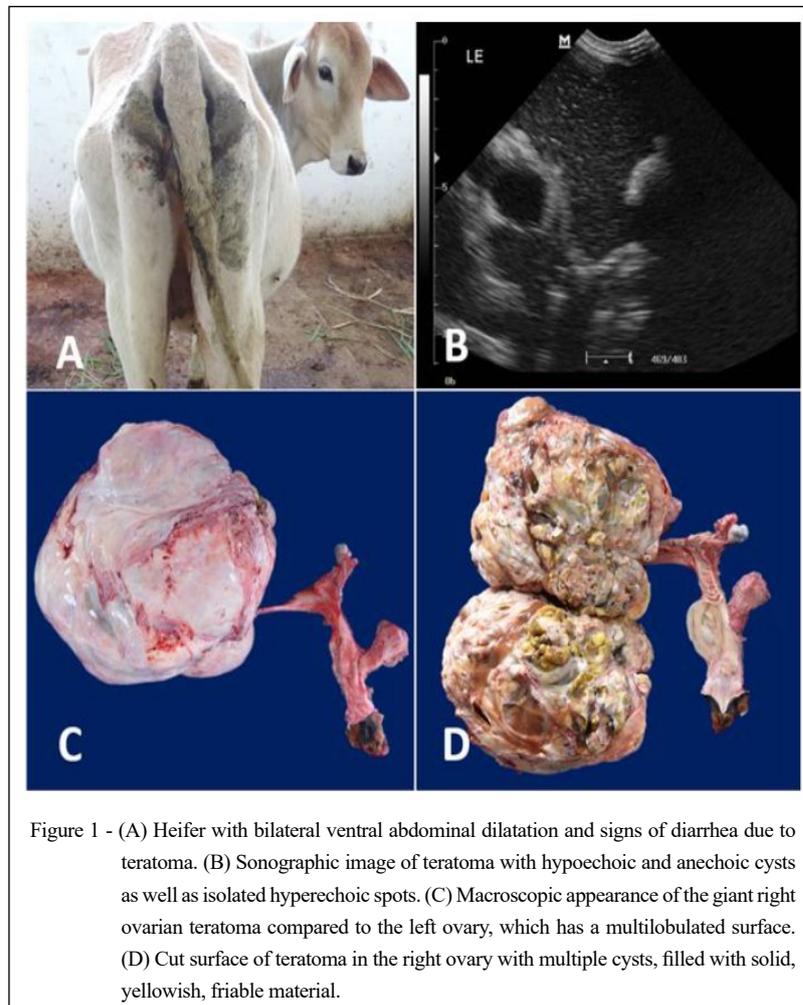


Figure 1 - (A) Heifer with bilateral ventral abdominal dilatation and signs of diarrhea due to teratoma. (B) Sonographic image of teratoma with hypoechoic and anechoic cysts as well as isolated hyperechoic spots. (C) Macroscopic appearance of the giant right ovarian teratoma compared to the left ovary, which has a multilobulated surface. (D) Cut surface of teratoma in the right ovary with multiple cysts, filled with solid, yellowish, friable material.

as enlarged mesenteric lymph nodes of up to 8 cm in diameter. On sectioning, the parenchyma of these lymph nodes demonstrated the presence of firm masses and fluid (edema). Small bowel loops (jejunum) had adhered to each other and to the omentum. Hence, both organs, the jejunum and the omentum, were in a congested state. The omentum had also adhered to the right ovary mass, which in turn had also adhered to the right abdominal wall, while the other organs remain unaltered. The fragments of all masses and internal organs were collected for analysis, and the samples were fixed in 10% buffered formaldehyde, routinely processed, and embedded in paraffin blocks for histological examination. Organ sections were cut at 4 μ m and stained with hematoxylin and eosin (HE) and Masson's trichrome.

Histopathological evaluation of the right ovary revealed complete loss of organ architecture, resulting from the proliferation of an unencapsulated neoplasm, consisting of different histological patterns. Cysts lined by an epithelial portion, consisting of a skin-like epithelium similar to that of the keratinized, stratified pavement type were identified. Adjacent to this epithelium were attached structures compatible with hair and hair follicles. Several nerve fascicles were identified underlying the epithelial tissue with low cell density, which were irregularly lined by perineuro-like tissue (Figure 2B). Other areas of the mass had secretory and ductal glandular structures, consisting of cells with basal and flattened nuclei. Moreover, structures compatible with sebaceous and sweat glands were near the large portions of mature hyaline cartilage or mineralized tissue, compatible

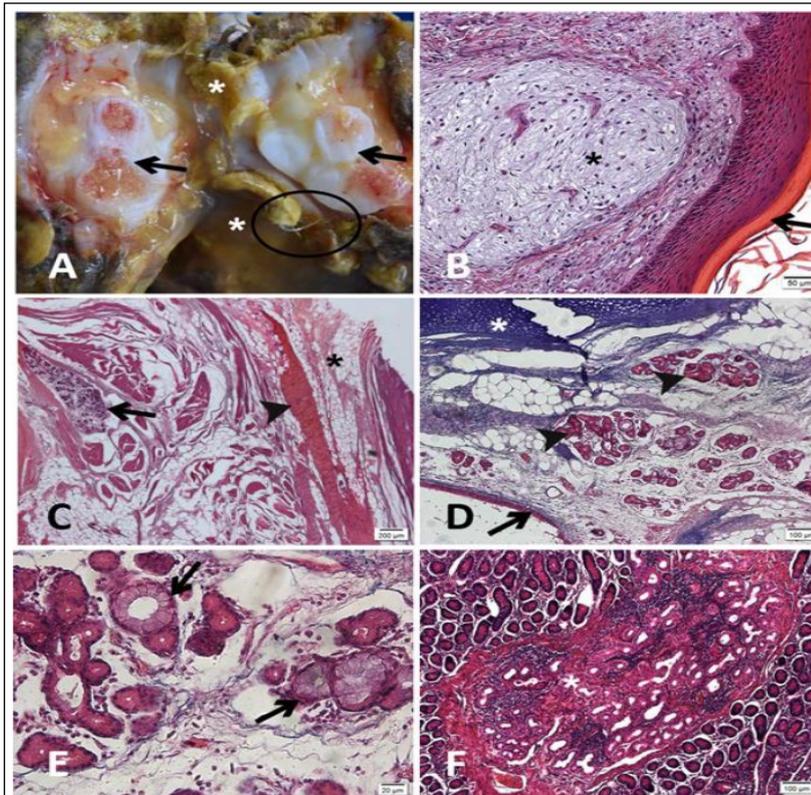


Figure 2 - Ovarian teratoma in a heifer. (A) Cut surface of the right ovary demonstrating cysts filled with solid, yellowish, friable material (asterisks). On the cyst wall, hair (circle) is visible. Adjacent to the cysts are multiple hard structures compatible with cartilaginous and bony tissue (arrows). (B) Teratoma cyst lined by keratinized stratified pavement epithelium (arrow) and adjacent nerve fascicles (asterisk). HE. Objective 20x; Bar= 50 μ m. (C) Teratoma area with abundant adipose tissue (asterisk), interspersed with bands of muscle tissue (arrowhead) and gland clusters (arrow). HE. Objective 4x; Bar= 200 μ m. (D) Teratoma cyst lined by pavement epithelium (arrow); adjacent to the cyst is adipose tissue, interspersed with gland clusters (arrowhead). At the top of the image, mature cartilage can be seen. Masson's trichrome. Objective 10x; Bar= 100 μ m. (E) Glands are seen in the middle of the tumor, consisting of tall, eosinophilic, and sometimes vacuolized columnar cells. Masson's trichrome. Objective 40x; Bar= 20 μ m. (F) The segment of the small intestine (jejunum), with the mucosa infiltrated by an aggregate of glands similar to those seen in ovarian teratoma, surrounded by fibrous connective tissue. Mononuclear inflammatory infiltrate (asterisk) is noted in the middle of the glands. HE. Objective 10x; Bar= 100 μ m.

with the bone matrix. Adipose and muscle tissues were also identified in the sample (Figures 2C-E). The left ovary presented complete loss of architecture due to several cystic areas surrounded by fibrous tissue. The areas of necrosis and inflammation consisting of neutrophils, macrophages, lymphocytes, and plasma cells were observed in both the ovaries.

The mesenteric nodules were markedly and diffusely infiltrated by macrophages in phagocytic activity and in epithelial cells with hyperchromatic nuclei. These cells also filled the lumen of lymphatic vessels. They had the same histological feature as that of the right ovary. In the intestinal serosa, infiltration of glandular ducts had

extended into the submucosa and mucosa, similar to that seen in the right ovary. These areas were also infiltrated by large numbers of lymphocytes and plasma cells, surrounded by fibrous connective tissue (Figure 2F). Hence, a diagnosis of ovarian teratoma was made, with infiltration of the lymph nodes, mesenteric vessels, greater omentum, and small intestine accompanied by secondary peritonitis.

The diagnosis of the present case was challenging for both the clinicians and pathologists during necropsy, since it is considered that volume increases in the abdominal cavity of cattles are usually associated with gestation or gastrointestinal disorders.

Loss of body mass, diarrhea, peritonitis, and adhesions were secondary signs and findings attributed to the presence of and compression exerted by the tumor within the abdomen. According to ARENSBURG et al. (2012), necrosis and fluid extravasation from the cysts into the abdominal cavity that occurs gradually as the teratoma grows, can trigger adhesion formation. Another contributing factor to these lesions is the appearance of pro-inflammatory cytokines in the peritoneum that reduces their fibrinolytic activity. All these phenomena may have contributed to the signs and lesions seen in this report, especially in a tumor of pronounced surface area and volume, which comprised 27.43% of the animal's entire body mass.

The presence of chronic mononuclear cell infiltration in the intestinal wall and the formation of adhesions may account for the incidence of diarrhea in this report. This sign, along with anemia and reduced body mass, are included in the paraneoplastic syndromes, even in the presence of appetite. Such presentation related to tumors stems from the presence of peptide hormones, growth factors, and cytokines released by neoplasms (MAZZANTE et al., 2019).

The studies by CÂMARA et al. (2016) and BUFFARINI et al. (2017) reported mild abomasal impaction, resulting from mechanical interference with pyloric drainage, intestinal obstruction, and death by hemoperitoneum as consequences of teratoma in a cattle. In the equine species, an abdominal teratoma caused severe acute colic due to strangulation obstruction of the small intestine, in addition to the enlargement of mesenteric lymph nodes, adhesions between the neoplasm and omentum, jejunum, cecum, mesenteric root, and abdominal wall, also with associated peritonitis (ARENSBURG et al., 2012). This condition appears in 10% of women with malignant teratomas, resulting from infection, torsion, or tumor rupture (TEWARE, 2000). In cattle with peritonitis, changes in vital

parameters can be identified, such as tachycardia, tachypnea, marginally elevated (39.2-39.5 °C) or normal rectal temperature, congested mucous membranes, decreased or absent rumen movements, and scanty stools. However, the vital parameters remained practically unchanged in our specimen, with no observation of any of the mentioned changes. Furthermore, according to SCOTT (2013), it is known that omentum-wrapped lesions can mask clinical signs associated with peritonitis.

On histopathological evaluation, the neoplasm showed tissue components of the mesoderm and ectoderm. In mice studies, BALAKRISHNAN & CHAILLET (2013) identified that one of the main mechanisms of teratoma formation occurred in a single germ cell due to genetic and cell signaling defects, completed meiosis phase I, and disrupted meiosis phase II, thence with development through parthenogenesis. These authors argued that the origin of such defects is in granulosa cells or oocytes. CARLUCCIO et al. (2017) considered that teratoma formation in cattles might begin in the third month of gestation, since this is when primary oocyte formation in the bovine fetus begins, which is completed between 80 and 130 days. This information, coupled with rapid tumor development, justifies the diagnosis of teratoma in a young animal, similar to that recorded in the literature.

Teratomas are usually incidental findings, which are rarely associated with clinical disorders in domestic animals (ARENSBURG et al., 2012). In cattles, early slaughter can be considered a limiting factor in the appearance of more significant lesions such as infiltrations and/or metastases. However, it is suspected that ovarian teratoma infiltration in these animals may be more frequent but is underdiagnosed or undiagnosed under abattoir conditions. The reduced space resulting from the mass and expansive growth against large cavitory organs such as the rumen and pre-stomach facilitates a more significant contact of these components with other adjacent abdominal structures, such as the intestine, predisposing to the incidence of infiltration by direct extension.

The presence of immature nervous and glandular tissue identified in the tumor suggested the diagnosis of immature ovarian teratoma. Furthermore, extensive necrosis (common in large neoplasms) and poorly differentiated and infiltrative tissues have been considered characteristics of immature teratoma in bovines (CARLUCCIO et al., 2017), as observed in the present case. In women, the amount of neuroepithelial tissue, which usually develops primitive nerve structures, results in proper

classification of immature ovarian teratomas (ABIKO et al., 2010; PAULINO JÚNIOR & PEDROSA, 2016). As in women, the immature teratoma in the heifer of the present study, acquired a malignant behavior characterized by infiltration of adjacent organs. This involvement of other systems, associated with necrosis, inflammation, and fibrosis, resulted in a severe gastrointestinal clinical disorder.

Teratomas must be differentiated from other ovarian-based tumors, such as dysgerminomas (another tumor originating from germ cells) and those developed from granulosa-theca cells that can cause anestrus, nymphomania, and masculinization, characterized histologically by irregular accumulations of granulosa cells separated by supporting stroma, and the presence of cysts (FARIN & ESTILL, 1993; AGNEW & MACLACHLAN, 2017). Dysgerminomas, usually affecting elderly animals, are highly cellular and metastasis-producing; however they consist of large, polyhedral cells with vesicular nuclei, similar to primitive germ cells, which are separated by connective tissue septa (JUNG et al., 2015; AGNEW & MACLACHLAN, 2017). Abscesses, hematomas, ovarian and para-ovarian cysts, as well as oophoritis, are considered non-tumor diagnoses (TROEDSSON & CHRISTENSEN, 2009). All these diagnoses were ruled out by clinical, macroscopic, and histopathological evaluation in our study.

Poorly differentiated ovarian teratomas can compromise cattle reproduction due to bilateral functional alteration of the ovaries. Moreover, internal organs are greatly affected due to their large dimensions, infiltration capacity, necrosis induction, and inflammation. This neoplasm should hence be considered in the differential diagnosis among heifers with the absence of estrus and in those associated with abdominal distension and with no history of gestation and gastrointestinal disorders accordingly.

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BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL DECLARATION

The authors of this article declare that the current data of the study was not submitted to the evaluation of the Ethics and Research Committee of the "Universidade Federal da Paraíba," but we are aware of the content of the Brazilian resolutions of the National

Council for the Control of Animal Experimentation (CONCEA) <<http://www.mct.gov.br/in-dex.php/content/view/310553.html>> if the study involves animals. This is a case report of a sick bovine animal brought by its owner to the Large Animal Clinic-UFPB and later referred to the Veterinary Pathology Laboratory-UFPB. Therefore, the procedures were necessary and part of routine clinical and diagnostic care. Hence, the authors take full responsibility for the data presented and are available for possible questions, should they be requested by the competent authorities.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interests. The funding sponsors had no role in the design of the study, collection, analyses, interpretation of data, writing of the manuscript, and decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally in the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version accordingly.

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