



Granulosa cells tumor in mules

Tumor das células da granulosa em mulas

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Abstract

Due to the current understanding of infertility and absence of ovarian activity in the female mule, scientific material regarding their reproductive tract is lacking. Information regarding evaluation of the reproductive system, as well as diagnoses of ovarian neoplasms, is scarce for female mules. The purpose of this paper is to report the first case of granulosa cell tumor (GCT) in a female mule, characterized through histology, which suggests evidence of cyclicity. During the breeding season, within the Southern hemisphere, mules' ovaries were obtained from slaughterhouses. Notably, a pair ovarian rated was remarkable in size (16.5 cm in diameter), weight (1.92 kg) as well as number of antral follicles. On sagittal section, the tissue was trabecular and fluid filled. Histology revealed irregular cyst formations full of amphophilic fluid ranging in color from pinkish to purple. The granulosa cells showed up in abnormal histology, featuring a granulosa cell tumor. The contralateral ovary was evaluated, demonstrating anatomy, weight and granulosa cells from normal. Therefore, we have described the first granulosa cells tumor report in a mule via gross and histological features, which suggests evidence of cyclicity in female mules.

Keywords: Ovarian Neoplasm. Histologic. Cyclicity. *Equus Mulus*.

Resumo

O conhecimento reprodutivo sobre as mulas está vinculado com a infertilidade e ausência da atividade ovariana, porém é notável a dificuldade na obtenção de material científico relacionado ao trato reprodutivo. As informações sobre a avaliação do sistema reprodutivo, bem como o diagnóstico de neoplasias ovarianas são escassas para estas fêmeas. O objetivo deste trabalho é relatar o primeiro caso de tumor das células da granulosa (TCG) em uma mula, caracterizando-o através da histologia, a qual sugere evidências de ciclicidade. Durante a estação de monta no hemisfério sul, foram obtidos pares de ovários muares provenientes de abatedouro. Notavelmente, um ovário do par avaliado, destacou-se no tamanho (16,5 cm de diâmetro) e peso

(1,92 kg), bem como no número de folículos antrais. Na secção sagital, o tecido apresentou característica trabecular e com conteúdo líquido. O exame histológico revelou formações císticas irregulares, com conteúdo líquido anofílico, variando entre a coloração rosa e roxo. As células da granulosa apresentaram-se anormais no exame histológico, caracterizando um tumor de células da granulosa. O ovário contralateral foi avaliado, demonstrando anatomia, peso e células da granulosa normais. Portanto, este trabalho descreve o primeiro relato de tumor das células da granulosa em uma mula, com características macro e microscópicas, embasando evidências de ciclicidade nessa fêmea muar.

Palavras-chave: Neoplasia de Ovário. Histologia. Ciclicidade. Equus Mulus.

Introduction

Mules are usually disregarded of the reproductive chain context, due to the classic quotes of infertility and absence of ovarian activity. However, the recent literature is scarce in respect to the information of the reproductive system, as well as for diagnoses of the possible ovarian neoplasms.

In mares, the granulosa cells tumor (GCT) is the most common neoplasm (Ellenberger et al., 2007; Foster, 2007), representing more than 85% of the breeding season tumors (McCue, 1998). However, in the Egyptian donkey, only one diagnosis of GCT was reported after an evaluation of 165 cases of breeding season tumors (Sokkar et al., 2001). In female mules, no information was found about the incidence or even of the existence of the tumor.

The GCT is derived of stromal and sex cord cells (Bosu and Smith, 1993) and it may be classified as solid, cystic or polycystic (McCue et al., 1991; Foster, 2007), although generally presented as polycyclic, with solid tissue areas (McCue et al., 2006). Most GCTs unilaterally present and are benign, although they may bilaterally presented (Frederico et al., 2007) in the malign form (Patrick et al., 2003). These tumors in all species have potential for hormone synthesis (McCue et al., 2006), leading to the appearance of different sexual behavior, such as the stallion behavior, continuous or intermittent estrous, and prolonged anestrus (Ellenberger et al., 2007; Gharagozlou et al., 2013).

The clinical diagnosis is made from history, inspection of animal behavior, the presence of unilateral ovarian enlargement associated with inactivity of the contralateral ovary and hormone ratings (McCue et al., 1991). Histologically, the tumor is characterized by cysts, suggesting a

disorganized follicle formation, with several layers of cells, similar to the granulosa cells within the follicular structures, and a supporting stroma, which may contain cells similar to theca cells (McCue et al., 2006).

The true incidence of GCT and other reproductive tumors may be grossly underestimated in female mules. Thus, the purpose of this paper is to report the first case of an ovarian granulosa cell tumor in a female mule, characterized morphologically through histology, ultimately suggesting evidence of cyclicity.

Case report

During the equine breeding season in the southern hemisphere, latitude 23°S, mules' ovaries ($n = 98$) were obtained at a local slaughterhouse. Among mules' ovaries that were obtained, one was distinguished by size, weight and number of antral follicles. This ovary averaged 16.5 cm in diameter, weighed 1.92 kg (analytical balance, Bel®, Monza-Italy) and demonstrated a macroscopically smooth surface with attached capsule (Figure 1A). The sagittal appearance of the two hemiovaries was trabecular, with net content filling every space (Figure 1C). In the region of the ovulatory cavity, there was a bleeding follicle with a diameter of 8 cm (Figure 1B). The contralateral ovary was also sagittally sectioned into two hemiovaries. This measured 4 cm and weighed 4.56 grams, contained follicles and a corpora lutea, having an overall normal ovarian characteristic.

The ovaries were fixed in 10% formalin, submitted for histology and examined under a light microscope (Nikon®, Tokyo-Japan). Histological staining was used PAS. Histological

analysis revealed abnormal tissues present within the neoplastic ovary. The neoplasm was characterized by irregular cystic formations filled with amphophilic fluid ranging from pinkish to purple in color. The cells were covered with multiple layers within the basal lamina and were predominantly palisading in pattern, which had become parallel and perpendicular to the basement membrane bounded together by fibrous stroma (Figure 1D). Such formations were well differentiated, with abnormal granulosa cells present within the ovarian follicles. In two of these structures, stronger cell proliferation was found in the presence of inclusion bodies called Call-Exner, which are small tubular arrangements containing eosinophilic material are found in GCT. Also, proliferation sites and thecal luteinization areas were found. Therefore, the tumor observed in this mule's ovarian tissue exemplifies a

well-differentiated GCT. The contralateral ovary had homogeneous appearance with connective tissue and the presence of blood vessels. This was a prime preantral follicles with normal paved granulosa cells surrounding the oocyte. Therefore, we considered that the contralateral ovary was not tumor

Discussion

This represents the first report of GCT in mule's ovarian tissue. The tumor was unilateral, measuring 16.5 cm in diameter and weighing 1.92 kg. The tumor in question is described as the most frequent ovarian tumor in mares (McCue et al., 2006), representing 2.5% of all neoplasms diagnosed (Sundberg et al., 1977). Our study group noted a GCT after evaluation of 98 mule

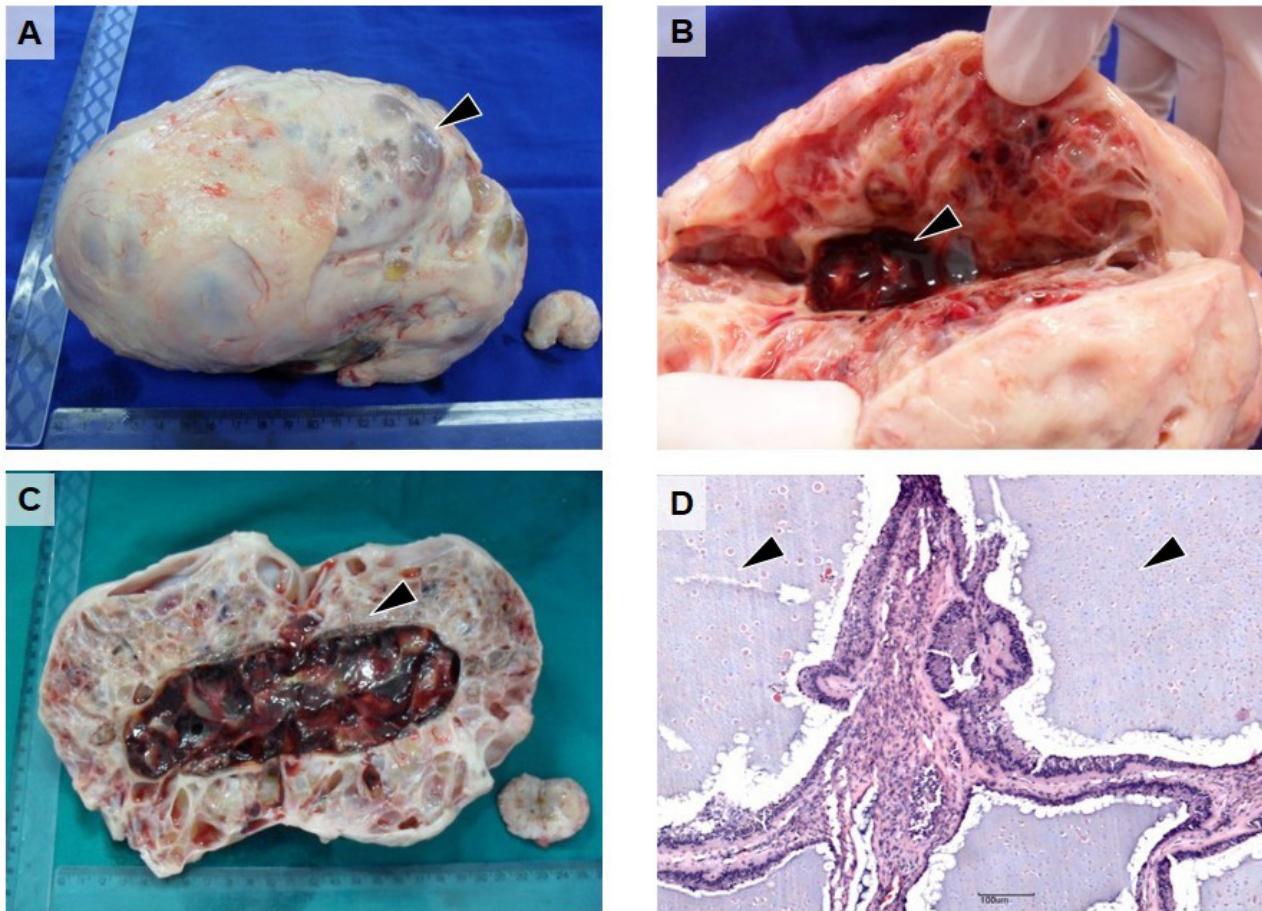


Figure 1 – A: Mule ovary with granulosa cells tumor (arrow) and contralateral ovary with normal morphometric characteristics; B: Bleeding follicle in the ovulatory cavity (arrow); C: Trabecular ovarian tissue; D: Multiple irregular cysts filled with amphophilic substance coated cell layers.

ovaries obtained from a slaughterhouse, a rough frequency of 1%. The presence of GCT in mules, similar to what occurs in mares, could have direct effect on cyclicity and reproductive behavior of female mules.

Mares diagnosed positive for the GCT often have large ovaries felt on rectal palpation, with loss of normal anatomy (Hinrichs and Hunt, 1990). This corroborates with the macroscopic finding of mule ovarian tumor observed in the present report. In cases of GCT, the contralateral ovary usually presents as small, firm and inactive due to a negative feedback mechanism acting on the hypothalamus and pituitary axis caused by hormones secreted by the GCT (Nelly et al., 1993). However, there are reports of mares with functional contralateral ovaries (McCue et al., 1991; Crabtree, 2011). In the mule, the contralateral ovary displayed normal anatomical and histologic characteristics.

The macroscopic architecture may vary from one GCT to another. In this case, the structure of the normal ovary was replaced by a tumor with a multicystic structure and numerous irregular trabeculae filled with liquid, similar to previous report by Crabtree (2011), McCue et al. (2006) and Ellenberger et al. (2007).

The most common histologic features observed in GCT in mares are benign neoplastic granulosa cells with a tubular and trabecular appearance (Ellenberger et al., 2007). This description corroborates our findings from the female mule GCT; however, the cells found were abnormal granulosa cells present in the ovarian follicles. Two of these structures showed marked cellular proliferation in the presence of Call-Exner corpuscles.

This study reports the presence of a granulosa cells tumor in a female mule. Macroscopic and histologic features observed were similar to granulosa cells tumor found in mares. Therefore, we can suggest evidence of cyclicity in female mules.

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