

# Histopathological and Immunohistochemical Characterization of Spontaneous Uterine Leiomyomas in Two Captive Asian Elephants

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## Abstract

Two captive female Asian elephants (*Elephas maximus*) in Thailand were euthanatized after prolonged recumbency, weakness, and unresponsive treatment. Necropsy revealed multinodular masses of 5-10 cm in diameter which were irregular, large, white and firm in consistency scattered throughout the uterine horns and uteri of both the elephants. Histologically, the tumors displayed mildly pleomorphic, spindle-shaped cells arranged in interlacing pattern interpreted as muscle bundles. The bundles of the muscle cells were interspersed by small amounts of collagen fibers and blood vessels. For immunohistochemistry, the tumor cells showed diffuse positivity for vimentin, desmin, and smooth muscle actin. Based on the pathological results, uterine leiomyomas were diagnosed. This study describes the morphology and immunohistochemical characteristics of the uterine smooth muscle tumor in Asian elephants.

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**Keywords:** Asian elephant, histopathology, immunohistochemistry, uterine leiomyomas

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## Introduction

Uterine leiomyomas are tumors arising from the uterine smooth muscle cells and the most common, benign tumors affecting more than 70% of gynecologic tumors in women in reproductive-age worldwide (Bulun, 2013; Nucci, 2013). In animals, tumors of the uterus have been reported in captive and wildlife animals, including cats, dogs, rabbit, cheetah, pot-bellied pigs, monkey and sheep (Andreasen and Mahaffey, 1987; Munday and Stedman, 2002; Miller et al., 2003; Walzer et al., 2003; Kurotaki et al., 2007; Corpa and Martinez, 2010; Long et al., 2010;). Because of subclinical condition, however, most of the lesions have been accidentally found in post-mortem studies (Hoffsis et al., 1986; Munday and Stedman, 2002; Siegal-Willott et al., 2005). Using the ultrasound examination, only a few reports have described the prevalence of uterine leiomyomas in large, endangered wildlife animal species such as Asian elephants (*Elephas maximus*) and Indian rhinoceroses (*Rhinoceros unicornis*) (Lueders et al., 2010; Hermes et al., 2014). Although there has been a report of uterine fibroleioma in an Asian elephant (Sapundzhiev et al., 2007), its detailed pathological and immunohistochemical characteristics have not yet been studied. In this report, we described the histological and immunohistochemical characteristics of uterine leiomyomas in two captive Asian elephants, and raised important questions of the female gynecologic tumors affecting breeding program, in order to sustain the population of this endangered animal species.

## Materials and Methods

Two female Asian elephants aged 15 years (Elephant 1) and 50 years (Elephant 2), from the Thai Elephant Conservation Center and a private elephant camp, respectively, were euthanatized after prolonged recumbency due to hind limb lameness, severe abdominal gas distension, and depression. These animals had no previous history of pregnancy, however, husbandry and reproductive management were monitored by authorized veterinarians. Neither reproductive hormone analysis nor rectal ultrasonography was performed during these study periods; therefore, reproductive status was unknown. Once a week prior to being euthanatized, blood samples were collected for hematology and blood chemistry tests to determine health status. Results indicated that both animals had marked leukocytosis and neutrophilia, and showed mild to moderate degrees of dehydration. In Elephant 2, serum creatine kinase (CK) was increased by 20 times, suggesting severe muscular degeneration and necrosis. Supportive treatments using oral administration of anti-inflammatory and antimicrobial drugs (phenylbutazone and ceftriazone, respectively) were done without any clinical improvements. After humane euthanasia using an overdosed intravenous injection of barbiturate drug, complete necropsies of both the animals were performed and selected specimens were submitted for further histological diagnosis.

Representative samples of the hearts, lungs, livers, kidneys, spleens, lymph nodes and uterine masses were fixed in 10% buffered formalin and embedded in paraffin blocks. Histological staining was done on 4- $\mu$ m-thick sections. Routine H&E staining and Masson's trichrome staining were performed as described elsewhere. The sections were also subjected to immunohistochemical staining using an avidin-biotin complex immunoperoxidase technique (Vector Laboratories Inc., Burlingame, CA) with the following primary antibodies: cytokeratin (AE1/AE3), vimentin (Diagnostic BioSystems, Pleasanton, CA), desmin, smooth muscle actin (SMA), S-100, proliferating cell nuclear antigen (PCNA), Ki-67 and C-kit (from Nichirei Bioscience, Tokyo, Japan). The nuclei were counterstained with Mayer's haematoxylin.

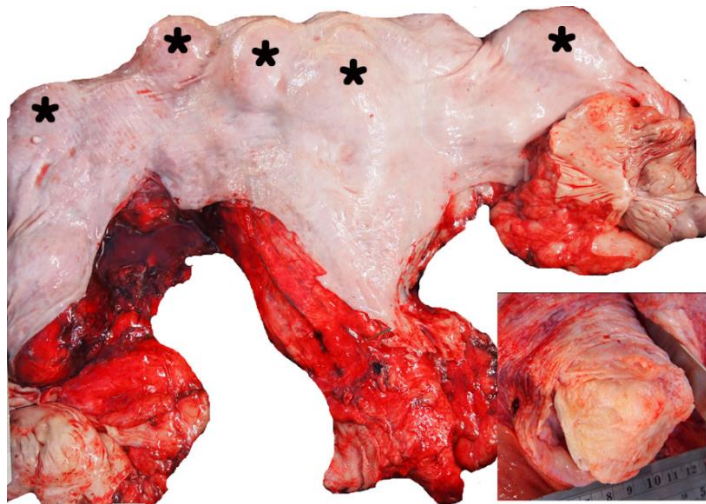
## Results and Discussion

Macroscopically, both the animals revealed emaciated body conditions. There were dark, red and firm in consistency of the lungs, with yellowish mucoid fluid presented in the tracheas. The livers of both the animals were congested. The inguinal lymph nodes were swollen and reddish in color (data not shown). The uteruses of both animals showed similar lesions, with obvious, irregular, large, multinodular masses sized 5-10 cm in diameter scattered throughout the uterine horns and the uterine bodies (Fig 1). The masses were firm in consistency and no metastasis was observed. No other gross findings were detected. Microscopically, the lungs of both animals revealed moderate degree of multifocal, suppurative bronchopneumonia. Multifocal fatty degeneration in the livers was found (data not shown). Tissue samples of the uteruses revealed poorly demarcated, unencapsulated masses displaying well-differentiated, long, plump spindle cells arranged in wavy patterns with large interlacing bundles of collagenous fibrostroma connective tissues (Fig 2A). The individual cells had mild to moderate eosinophilic cytoplasm; hyperchromatic, elongated blunt-ended nuclei; and discernable nucleoli, with rare observation of mitotic activity (Fig 2A). The tumor cells were stained bright red with Masson's trichrome stain, while the stromal connective tissues were stained dark blue (Fig 2B). Immunohistochemically, the cytoplasm of the tumor cells was intensely and diffusely labeled by specific markers for vimentin, desmin, and SMA (Fig 3). No expressions of cytokeratin (AE1/AE3), S-100, and C-kit were found. The PCNA and Ki-67 were expressed by some nuclei of the neoplastic cells (Fig 3). Based on gross and histological examinations, uterine leiomyomas were diagnosed in both the elephants.

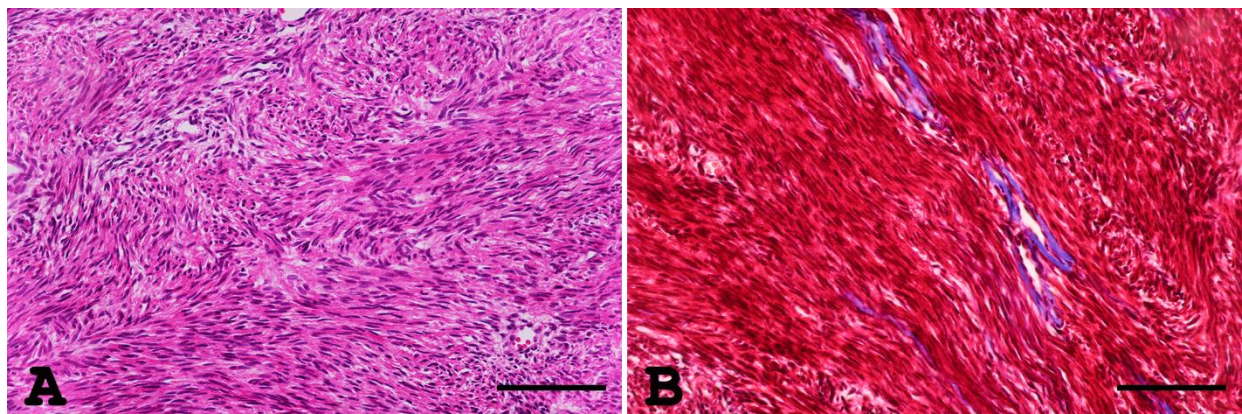
Uterine leiomyoma is the most common gynecologic, smooth muscle tumor in reproductive-age women worldwide (Nucci, 2013). Although it is the most common gynecologic tumor, little is known about its etiology and genetic or contributory factors that lead to the development of uterine leiomyomas (Backlin et al., 2003; Bulun, 2013; Medikare et al., 2011). In the large mega-herbivores like elephants and rhinoceroses, uterine leiomyoma is usually associated with age and non-reproductive status in which the cystic uterine hyperplasia might also occur with leiomyomas

(Hermes et al., 2004; Hildebrandt et al., 2006). Although tumor of the elephant uterus has been previously reported, neither special stain nor immunohistochemical analysis was performed (Sapundzhiev et al., 2007). In the present study, tumor cell morphology and the patterns of immunohistochemical stainings of uterine tumors were found to be similar to the uterine smooth muscle tumors in cats, dogs and humans (Andreasen and Mahaffey, 1987; Miller et al., 2003; Nucci, 2013). These elephants were in the reproductive age (15 years old) and post-reproductive age (50 years old), and had no previous history of pregnancy. The elephants showed subclinical signs, but it should be noted that these tumors can affect the fertility in reproductive-age elephants, which normally is not found in young female like one of our studied elephant. It should be taken into account that multiple mating without conception in many young female elephants might be

one of the causes of this tumor (Hildebrandt et al., 2006). Thus, breeding at an age before 15 years old may increase the conception rate, and also reduce the incidence of uterine leiomyoma (Hildebrandt et al., 2006). Although the mechanism of uterine leiomyoma formation remains unclear, previous studies have pointed out that a hyperestrogenic state, or an increase in estrogen and progesterone receptors, is relevant to the initiation of tumors (Bulun, 2013). Because of their hormonal dependency, these tumors may be targeted by environmental chemicals, and the biologic effects of this are mediated through the estrogen and progesterone receptors (Rein et al., 1995). Nonetheless, it has been hypothesized that endocrine-active compounds such as bioaccumulation of pesticides are also related to an increase in the formation and development of these tumors (Keymer et al., 1988; Hodges et al., 2000).

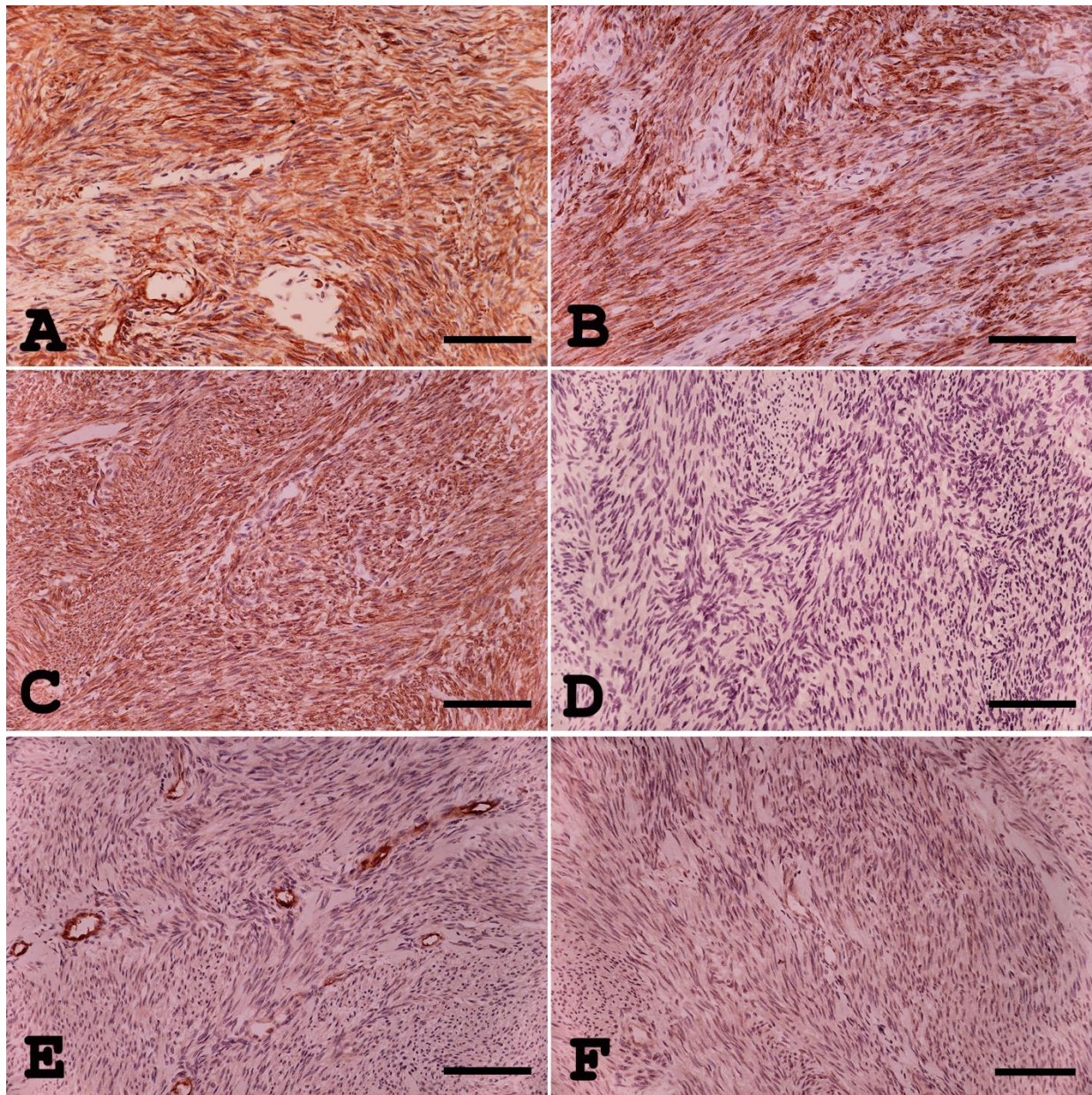


**Figure 1** Uterus of Elephant 2 showing multinodular, white, firm masses (inset) sized 5-10 cm in diameter (\*) scattered throughout the uterine body and the uterine horns.



**Figure 2** The uterine mass from the elephant in Figure 1 displayed densely packed, well-differentiated long, plump spindle cells arranged in wavy patterns with large interlacing bundles of collagenous fibrostroma connective tissues (A) (H&E stain, bar = 200  $\mu$ m). The tumors cells were stained bright red, while stromal connective tissues stained blue (B) (Masson's trichrome stain, bar = 200  $\mu$ m).





**Figure 3** Immunohistochemistry of the masses in Figure 1 revealed diffuse positivity for vimentin (A), desmin (B), and SMA (C), while it was negative for cytokeratin (D), S-100 (E) and C-kit (F) (Immunohistochemistry, DAB, bar = 100  $\mu$ m).

It should be noted that birth rate in Asian elephants is on the decline, and this might be a major concern as regards the decreasing population of captive Asian elephants in Southeast Asia (Thitaram et al., 2009). It has been suggested that inappropriate management such as quality of feed may play a role in the current low birth rate in captive Asian elephants (Thitaram et al., 2009). In addition, exposure to pesticides in captive elephants in Thailand has increased considerably due to habitat loss and a situation that offers decreased natural food source. Therefore, ante-mortem incident monitoring via hormonal and reproductive examination is critical to sustainable conservation of this endangered animal species.

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## บทคัดย่อ

### ลักษณะทางจุลพยาธิวิทยาและภูมิคุ้มกันฮิสโตเคมีของเนื้องอกชนิดไลโอไมโอมาที่เกิดขึ้นเองในมดลูก ของช้างเลี้ยงสายพันธุ์เอเชีย 2 เชือก

กฤษฎาภรณ์ พริ่งเพราะ<sup>1,2\*</sup> อีโรโอะมาดาระมา<sup>3\*</sup> จิราภรณ์ ศรีทัน<sup>2</sup> ปิติกายจน์ บำเพ็ญพล<sup>4</sup> ภาณุณี เพ็ชรศรี  
เฉลิมชาติ สมเกิด<sup>5,6,7</sup> ฉัตรโชติ ทิตาราม<sup>5,6,7</sup>

ช้างเลี้ยงเอเชีย (*Elephas maximus*) จำนวน 2 เชือกในประเทศไทยถูกการุณยฆาตหลังจากแสดงอาการป่วยล้ม ลูกไม่ได้มาเป็นเวลานาน และไม่ตอบสนองต่อการรักษา จากการชันสูตรซากของช้างทั้ง 2 เชือก พบก้อนเนื้องอกสีขาวขนาดเส้นผ่านศูนย์กลางประมาณ 5-10 เซนติเมตร ลักษณะแน่น ผิวขรุขระกระจายอยู่ในบริเวณมดลูกและปีกมดลูกทั้ง 2 ข้าง ลักษณะทางจุลพยาธิวิทยาชี้ให้เห็นว่าเซลล์เนื้องอกมีรูปร่างยาวรีคล้ายรูปกระสวย มีเซลล์หลายขนาด แทรกด้วยเนื้อเยื่อเกี่ยวพันและหลอดเลือด เมื่อตรวจเนื้องอกด้วยวิธีภูมิคุ้มกันฮิสโตเคมี พบว่าเนื้องอกของช้างทั้ง 2 เชือกให้ผลบวกต่อแอนติบอดีชนิดไวนเมนติน เดสมิน และสมุทมัสดเซลล์แคคติน จากผลการชันสูตร วินิจฉัยว่าเป็นเนื้องอกของมดลูกชนิดไลโอไมโอมา การศึกษานี้รายงานลักษณะทางจุลพยาธิวิทยาและภูมิคุ้มกันฮิสโตเคมีของเนื้องอกมดลูกในช้างเลี้ยงสายพันธุ์เอเชีย

**คำสำคัญ:** ช้างเอเชีย จุลพยาธิวิทยา ภูมิคุ้มกันฮิสโตเคมี เนื้องอกชนิดไลโอไมโอมา

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