# SIMULTANEOUS UTERINE LEIOMYOMA AND ENDOMETRIAL HYPERPLASIA IN A WHITE-NOSED MONKEY (*CERCOPITHECUS NICTITANS*). FIRST CASE REPORT

Leiomioma uterino e hiperplasia endometrial simultáneos en un cercopiteco de nariz blanca (*Cercopithecus nictitans*). Primera descripción de un caso.

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**ABSTRACT**

This paper describes histopathological and immunocytochemical features of a combined uterine leyomioma and a non atypical complex endometrial hyperplasia in a white-nosed monkey (*Cercopithecus nictitans*). Immunocytochemically, uterine leiomyoma was α-actin positive, and negative for desmin. By the other hand, endometrial hyperplasia showed strong immunoreaction against ciclin D1, cyclooxygenase-2 (COX-2), oestrogen receptor, isoform A of progesterone receptor and slight p53 immunoreaction. This is the first immunocytochemical description of an endometrial hyperplasia in a white-nosed monkey. This lesional spectrum, similar to those described in human pathology, suggests similar pathogenic mechanisms.

**Key words:** endometrial hyperplasia, leiomyoma, immunocytochemistry, *Cercopithecus*.

**RESUMEN**

El presente trabajo describe las características histopatológicas e inmunocitoquímicas de un leiomioma uterino simultáneo con una hiperplasia endometrial compleja no atípica en un cercopiteco de nariz blanca (*Cercopithecus nictitans*). Inmunocitoquímicamente, el leiomioma fue α-actina positivo y desmina negativo. La hiperplasia endometrial fue fuertemente positiva a la ciclina D1, a la ciclooxigenasa-2 (COX-2), al receptor

de estrógenos, a la isoforma A del receptor de progesterona y débilmente positiva a la proteína p53. Se trata de la primera descripción del espectro inmunocitoquímico de un caso de hiperplasia endometrial en un cercopiteco de nariz blanca. La similitud del cuadro lesional presentado con el descrito en la especie humana sugiere un mecanismo patogénico común.

**Palabras clave:** hiperplasia endometrial, leiomioma, inmunocitoquímica, *Cercopithecus*.

## INTRODUCTION:

Reports of tumours of the reproductive tract in non-human female primates are infrequent. To the best of our knowledge, very few cases are described in the literature, and include cervical and uterine leiomyoma (Beniashvilli, 1989) uterine leimyosarcoma (Cook *et al.*, 2004), adenomyoma (Hamerton, 1933), uterine carcinoma (Beniashvilli, 1989), tumours of the endometrial stroma (Toft and McKenzie, 1975) and fibrothecoma (Grahan and McClure, 1977). These descriptions are interesting because, although reproductive biology and endocrinology of old world non-human primates are similar to those of human females and are likely to be an appropiate experimental model of reproductive biomedical research (Cline *et al.*, 2001), there are some differences (anovulatory first menstrual cycles, seasonal variations in reproductive cycles), which facilitate studies to establish phylogenetic differences between reproductive phisiology in monkeys and humans (Daylei *et al.*, 1981; Resko *et al.*, 1982).

Uterine leiomyoma is the most common uterine tumour described in humans (Crum *et al.*, 2004). It is a benign smooth muscle neoplasm, which is frequently found in approximately 50% of fertile women. In domestic animals, leiomyomas of the genitalia occur far more frequently in older reproductive females, and uterine leiomyoma is not as common as vaginal or cervical leiomyomas (Brodey and Roszel, 1967). This neoplasia is a monoclonal tumour, and the neoplastic transformation of myometrium to leiomyoma likely involves somatic mutations of the normal myometrium and a complex interaction of sex steroids and local growth factors (Maruo *et al.*, 2004). On the other hand, endometrial hyperplasia represents a non-physiological and non-invasive proliferation of endometrial glands of irregular size and shape with an increase in the gland/stroma ratio compared to the proliferative endometrium. The correct identification and classification of endometrial hyperplasia is important because some forms of hyperplasia are closely related to endometrial adenocarcinoma (Horn *et al.*, 2007), which are apparent in precursor lesions. This report describes the pathologic and immunohistochemical features of a uterine leiomyoma and a non atypical complex endometrial hyperplasia in a female white-nosed monkey (*Cercopithecus nictitans*).

## MATERIAL AND METHODS:

The animal was referred to the Clinic Veterinary Hospital (CEU-Cardenal Herrera University) from a wildlife recovery centre located in Valencia (East Spain). The animal (12 years-old and 4.8 kg in height) presented a history of chronic anorexia and diarrhea but did not respond to antibiotics, although *Salmonella spp.* was isolated from the fecal samples. The animal's health gradually deteriorated over 3 months with natural death occurring. No abnormal reproductive behavior had been observed in this animal. No records were available to confirm or refute whether this animal had ever given birth.

After post-mortem examination, samples from several organs (liver, spleen, intestine, kidney, lungs, heart, CNS, uterus, ovaries) were routinely collected and fixed in 10% formaldehyde in phosphate-buffered saline (Panreac

Chemicals, Castellar del Vallés, E-08211, Barcelona, Spain). Samples were paraffin-embedded, sectioned (4µm) and stained with hematoxylin and eosin (HE), Masson´s trichrome (Masson´s trichrome Goldner Kit, BioOptica Milano S.p.A., Milano 20131, Italy), and the avidin-biotin-peroxidase complex (ABC) using the following primary antibodies (Dako Diagnósticos, Barcelona, E- 08960 Sant Just Desvern. Barcelona, Spain): anti-Ki67 protein (clone MIB-1), anti-cyclooxygenase-2 (COX-2) (clone CX-294), anti-cyclin-D1 (clone DCS-6), anti-p53 protein (clone DO-7), anti-progesterone receptor-A (clone PgR 636), anti-estrogen receptor (clone 1D5), anti α-smooth muscle actin (colne 1A4) and desmin (clone D33). The immunoreaction was revealed with 3-3´ diaminobenzidine (Dako Diagnósticos) and counterstained with Harris´s hematoxylin (Across Organics, Geel, B-2440, Belgium) in order to differentiate the cellular nucleus.

**RESULTS:**

At necropsy, macroscopic signs of catarrhal enteritis, an hepathic cyst and severe chronic nefritis were observed. The uterus was enlarged, with its lumen partially occluded by a firm and whitish mass, with a firm white whorled cut section. Distension of the uterine lumen cranial to this mass contained a pale and viscous liquid, with multiple edematous endometrial polyps occluding part of the lumen (Figure 1). The uterine serosa was partially adhered to the antimesenteric border of the small intestine. No macroscopic alteration was observed in the ovaries. On basis of the macroscopic observations, a presumptive diagnosis of endometrial hyperplasia and uterine leiomyoma was carried out.

Histopatologically, a severe chronic interstitial nephritis was diagnosed, so the probably cause of death was chronic renal failure. By the other hand, microscopical examination of both ovaries showed multiple primary follicles, some of them calcified, and a small corpus luteum located in the left ovary.

The endometrial glands were highly irregular in both size and shape. The glands showed an increased structural complexity, with outpunchings and folds, with a gland-stroma ratio more than 2:1. Some glands were highly dilated and filled with an eosinophilic substance (Figure 2). Cytologically the glandular epithelium resembles a proliferative epithelium, with columnar cells with a clear cytoplasm with a rounded nucleus and prominent nucleolus with the same orientation to the underlying basement membrane. No signs of atypia or mitosis were observed. Additionally, no signs of endometriosis were observed in uterine serous membrane. According with the World Health Organization (WHO) classification (Silverberg *et al.*, 2003), the histopathological features were compatible with a non-atypical complex endometrial hyperplasia. In order to differentiate this pathologic diagnosis from other physiological situations (i.e. physiological hyperplasia due to estrous cycle), an immunohistochemical differentiation was carried out.

Some epithelial cells were labelled with the Ki-67 monoclonal antibody (cellular proliferation factor). Cyclooxigenase-2 (COX-2) is an enzyme that catalyzes the formation of a variety of eicosanoids that includes prostaglandins (Graham and McClure, 1977), and its overexpression, detected in cervical carcinomas (Ryu *et al.*, 2000), was strongly expressed in 100% endometrial and glandular epithelium (Figure 3). Cyclin D1 (cell-cycle regulatory protein whose upregulation is related with high variety of tumors (Dubois *et al.*, 1998) was overexpressed in approximately 90% of epithelial nuclei (Figure 4). By the other hand, p53 (cell-cycle regulator protein which usually acts as tumoral supressor factor, but mutations in its structure can lead to acts as a tumorogenic factor) expression was also detected in aproximately 5% of endometrial and glandular epithelium (Figure 5). Additionally, 100% of epithelium,

and more than 80% of stromal cells expressed the isoform A of progesterone receptor (Figure 6), and immunolabellig for oestrogen receptors showed similar immunostain distribution. On basis of histopathological and immunohistochemical results, a definitive diagnosis of complex endometrial hyperplasia was made.

By the other hand, histopathological examination of the uterine mass revealed a homogeneus population of densely packed spindle cells with undistiguishable cytoplasmatic borders, a strongly eosinophilic cytoplasm and a cigar-shaped heterochromatic nucleus (Figure 7). These cells were arranged in broad interlacing fascicles, some of which were intersected at a 90º angle, and were surrounded by a moderate amount of blood vessels. No mitotic figures were detected. The fascicles of the neoplastic cells presented a typical staining pattern with Masson´s trichrome, showing fascicles of

Figure 1: **Reproductive tract of a white nosed-monkey (Cercopithecus nictitans). The uterine lumen was partially occluded by a firm and whitish mass (asterisk), with a firm, white whorled cut. Cranial to this mass, multiple edematous endometrial polyps were observed occluding part of the lumen (arrows).**

Figure 2: **Microscopic view of the endometrial hyperplasia of white-nosed monkey of Figure 1. Note the complexity of some glands of the endometrium (asterisk) with outpouchings and folds. Gland:stroma is more than 2:1. Some glands were also dilated and filled with an eosinophilic substance (head arrows). Hematoxilyn and eosin stain .Bar: 200µm.**

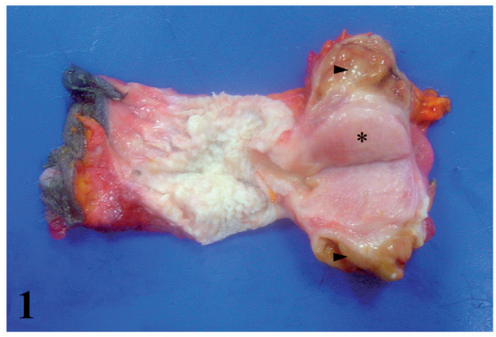
Figure 3: **Immunocytochemical expression of cyclooxigenase-2 (COX-2) in glandular (asterisk) and endometrial (head arrows) epithelial cells. 100% of cells are positive for immunolabelling. Avidin-biotin complex (ABC), anti-cyclooxygenase-2. Bar: 50µm.**

Figure 4: **Immunocytochemical expression of cyclin D1 in glandular (asterisk) and endometrial (head arrows) epithelial cells. Approximately, 90% of epithelial cells are positive for immunolabelling. Avidin-biotin complex (ABC), anti-Cyclin-D1. Bar: 50µm.**

Figure 5: **Immunocytochemical expression of p53 protein in endometrial epithelium. Positive cells (head arrows) showed nuclear immunostaining. Avidin-biotin complex (ABC), anti-p53 protein. Bar: 25µm.**

Figure 6: **Immunocytochemical expression of the isoforme-A of progesterone receptor. 100% of epithelium (head arrows), and more than 80% of stromal cells expressed positive nuclear immunostaining. Avidin-biotin complex (ABC), anti-isoforme A of progesterone receptor. Bar: 50µm.**

Figure 7: **Microscopic view of the uterine mass of white-nosed monkey in Figure 1. The image shows a homogeneous population of densely packed spindle cells with undistinguishable cytoplasmatic borders, a strongly eosinophilic cytoplasm and a cigar-shaped heterochromatic nucleus. These cells were arranged in broad interlacing fascicles, some of them intersected 90-degree-angle,surrounded by moderate numbers of blood vessels (head arrows). Hematoxilyn and eosin stain. Bar: 100µm.**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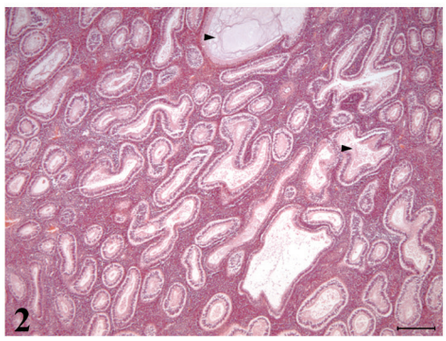
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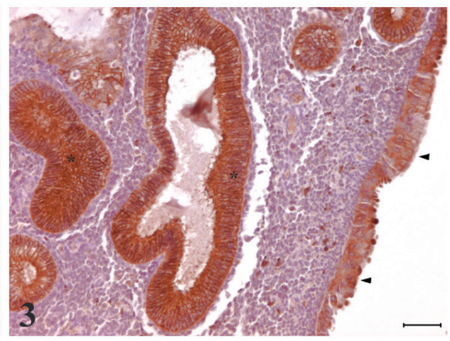
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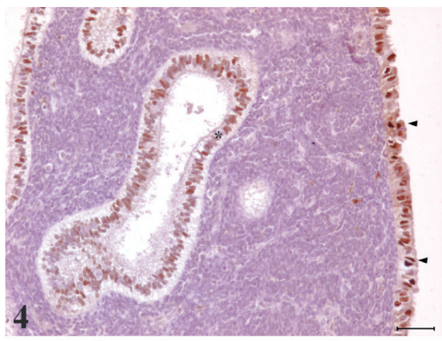
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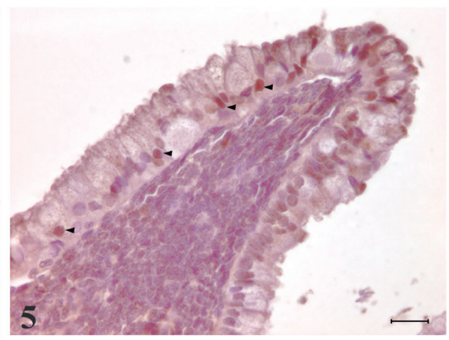
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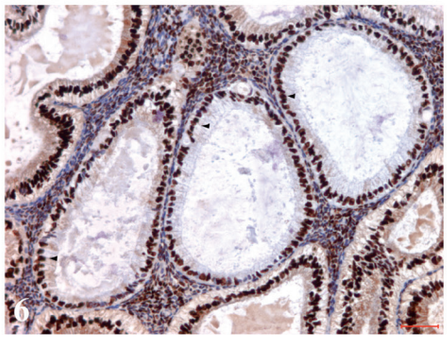
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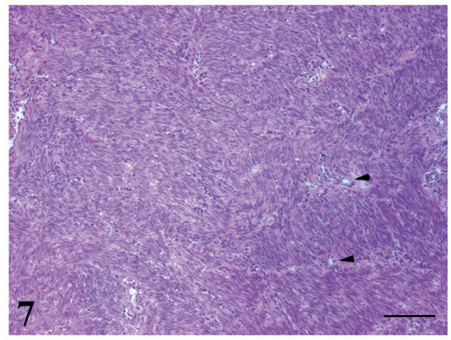
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the neoplastic cells surrounded by moderate amounts of well-vascularized connective tissue. Neoplastic cells expressed α-actin and were negative for desmin. According with the WHO standards[29], the diagnosis of well-differentiated uterine leyomioma was made.

## DISCUSSION:

The WHO classifies endometrial hyperplasias according with architectural alterations related with glandular complexity, amount of stroma whithin the glands and presence or absence of nuclear atypia, are related with oestrogen-derived type I endometrial carcinoma (Bokham, 1983). On basis of these features, endometrial hyperplasias are classified into simple (previously termed "cystic") which is characterized by proliferating glands with irregular shapes and signs of pseudoestratification with abundant stroma, and complex (previously termed "adenomatous") endometrial hyperplasia, with more densely crowded glands with minimal amount of stroma (Horn *et al.*, 2007). The term atypia is referred to loss of nuclear polarity, an euchromatic nucleus with prominent nucleolus, true glandular estratification and presence of mitotic figures can be present in simple (extremely rare) and complex endometrial hyperplasia. The risk of progression of endometrial hyperplasia into endometrial carcinoma is very closely related to the presence of cytologic atypia and architectural glandular crowding (endometrial glands closely packed with a prominent back-to-back position) (Horn *et al.*, 2007). On basis of this classification, our findings are consistent with a non-atypical complex endometrial hyperplasia.

Cyclooxigenase-2 is an enzyme that catalyzes the formation of a variety of eicosanoids that includes prostaglandins (Dubois *et al.*, 1998), and its overexpression has been detected in cervical carcinomas (Ryu *et al.*, 2000). Although COX-2 is constitutively expressed mainly in lumenal epithelium during menstrual cycle in the baboon and its expression in epithelium may be progestin-related (Kim *et al.*, 1998), a relationship between overexpression of COX-2 both in lumenal and glandular epithelium in endometrial hyperplasia and an early step in carcinogenesis has been proposed (Orejuela *et al.*, 2005). By the other hand, the high expression of Cyclin D1 either in lumenal and glandular epithelium has been found in human cases of complex endometrial hyperplasia (Quddus *et al.*, 2002), and p53 expression has only be detected in complex endometrial hyperplasia and endometrial carcinoma (Hachisuga *et al.*, 1992). These results are according with our observations, and support the diagnosis of complex hyperplasia. As far of our knowledge, there are no studies about immunohistochemical expression of cell-cycle regulatory proteins in cases of endometrial hyperplasia in primates. In human pathology, endometrial hyperplasia is closely related to endometrial carcinomas, and the expression of both proteins suggests a role in endometrial carcinogenesis, and may indicate mutagenic changes in proliferative cells (Hachisuga *et al.*, 1992). Progesterone is a key hormone in the endometrium that opposes estrogen-derived growth. Thus, insuffient progesterone will result in unopposed estrogen action that could lead to the development of endometrial hyperplasia and adenocarcinoma (Kim and Chapman-Davis, 2010). Endometrial hyperplasia is tipically oestrogen-derived lesion (Brodey and Roszel, 1967), and has been described experimentally in rhesus monkey (Baskin *et al.*, 2002). Thus, histologycal evidences observed in the ovaries on this case suggest secretion of oestrogens during folliculogenesis, and might be involved in the pathogenesis of the endometrial hyperplasia described in our case. By the other hand, recent reports suggest that overexpression of isoform A of progesterone receptor could leads to endometrial proliferation, hyperplasia and atypia (Fleisch *et al.*, 2009). Taking account these observations, the overexpression of the isoform A of progersterone

receptor observed in our case might be also envolved in the pahogenesis of endometrial hyperplasia.

Histopathological and immunohistochemical features of uterine leiomyoma in our case are similar to those described in other non-human primates, such as chimpanzee (*Pan troglodytes*) (Silva *et al.*, 2006), and is a common feature in aged animals (Beniashvilli, 1989). Although ovarian oestrogens are considered a primary promoter of leiomyoma tumorigenesis, the clinical and laboratory evidence to date would appear to indicate that progesterone may be important as a promoter of leiomyoma growth (Maruo *et al.*, 2004).

In summary, this is the first histopathological and immunohistochemical description of an uterine leyomioma combined with a complex endometrial hyperplasia in a white-nosed monkey. Similarities of immunohistopathological features with human beign suggest similar pathobiology.

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