# Morphology of the uterotubal junction of the cheetah (*Acinonyx jubatus*)

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**Abstract**

Less than 7000 cheetahs survive in the wild. Captive breeding has proved notoriously difficult. The uterotubal junction acts as major barrier and regulator to the passage of sperm. This study describes the morphology of the uterotubal junction of the cheetah. Reproductive tracts were obtained from 7 cheetahs that succumbed from natural causes or were euthanased for humane reasons. The uterotubal junction was isolated and examined macroscopically and microscopically. The extramural isthmus made a characteristic 90° bend before entering the mesometrial border of the uterus close to its tip. The intramural isthmus had approximately 4 primary folds lined by non-ciliated cuboidal to low cuboidal epithelium. The *Tunica muscularis* was robust, merged with its uterine counterparts and consisted of an inner circular layer and outer longitudinal smooth muscle layer. The uterine ostium opened via a muscular and glandular papilla which projected from the apex of the uterine lumen. A sharply demarcated circular zone of simple columnar epithelial cells surrounded the uterine ostium and separated the simple cuboidal to low cuboidal cells of the isthmus and uterine epithelium from each other. Branched tubulo-alveolar glands, some  with dilated lumens , were present in the papilla and sometimes extended into the adjacent endometrium. These glands might act as sperm storage areas, and could easily be confused with cystic endometrial hyperplasia. Low transverse endometrial ridges surrounded the papilla and extended caudally for a short distance before disappearing. The uterine glands were lined by a simple cuboidal epithelium.


**Keywords:** cheetah, uterotubal junction, papilla, isthmus; uterine ostium; branched tubulo-alveolar glands

**Introduction**

Cheetahs are listed as vulnerable by the International Union for Conservation of Nature with their total worldwide population estimated at 6674 individuals (IUCN, 2017). Ongoing habitat destruction, fragmentation, human animal conflict and predation threaten the survival of the wild cheetah population (O'Brien *et al.*, 1985; Marker *et al.*, 2003; Brown, 2011). Zoos and wildlife parks are tasked with maintaining a genetically healthy population as insurance against catastrophic extinctions (Brown, 2011). In contrast to reproduction in the wild, breeding of cheetahs in captivity has proved notoriously difficult (O'Brien *et al.*, 1985; Brown, 2011), with only a handful of facilities achieving reasonable success (Marker & O'Brien, 1989).

Cats and cheetahs are both primarily induced ovulators that sometimes experience spontaneous ovulations (Shille *et al.*, 1983; Asa *et al.*, 1992; Brown *et al.*, 1996; Gudermuth *et al.*, 1997; Lawler *et al.*, 1993). Sperm therefore need to be stored and preserved in the reproductive tract until ovulation occurs, which in the cat can be up to 32 hours post-coitus (Shille *et al.*, 1983). In cats the sperm are initially stored in the region of the uterotubal junction (UTJ) and the uterus, bound to the surface epithelium of the endometrium and inside the uterine glands (Chatdarong *et al.*, 2004). This is similar to the situation in the dog (Karre *et al.*, 2012). Shortly before ovulation a privileged portion of sperm is allowed to move through the UTJ, and the functional reservoir of sperm in the domestic cat is then found in the isthmus of the uterine tube (Chatdarong *et al.*, 2004).

The exact mechanisms of viable sperm storage in vertebrates are still poorly understood (Holt & Lloyd, 2010). Local signalling between sperm and the epithelial cells to which they are attached, as well as input by the ovarian hormones, may result in the epithelium maintaining a specific biochemical environment conducive to long term sperm survival. Likewise, the exact mechanisms of sperm release from storage remain obscure but are likely linked to the reproductive axis (Holt & Lloyd, 2010).

The UTJ, which links the uterine tube and uterine horn, has been defined as the region incorporating the uterine part of the isthmus and / or caudal last few millimetres of the extramural isthmus, and the cranial tip of the uterine horn (Hook & Hafez, 1968). This region has been shown to act as a major barrier to the passage of sperm in pigs (Mburu *et al.*, 1996), dogs (Karre *et al.*, 2012) and cats (Chatdarong *et al.*, 2004). Highly selective mechanisms guard the entrance to the UTJ ensuring that only a small proportion of sperm ever enter it (Holt & Fazeli, 2016). In pigs, the oedematous processes of the UTJ prevent the majority of sperm entering the isthmus; however, a mucous plug in the caudal isthmus may further force sperm into the diverticula of the region until dissolving just prior to ovulation (Hunter, 2002). Molecular recognition systems may also play a role in limiting sperm entry into the uterine tube (Holt & Fazeli, 2016). Sperm migrating towards the uterine tube are therefore

temporarily prevented from entering it by both mechanical and molecular barriers prior to ovulation, making the UTJ arguably one of the most important regions of the reproductive tract.

The morphology of the UTJ of 8 mammalian species (dog, rabbit, rat, sheep, pig, cow, pig-tailed monkey, rhesus monkey and rat) has been described in detail (Hook & Hafez, 1968) and variations between species and between individuals of the same species noted. The basic morphology of the uterine tube of the domestic cat has been described, although a description of the UTJ was lacking (Bansal & Varinder, 2008). The authors were unable to locate a detailed description of the UTJ of the domestic cat despite an extensive review of the literature. Nothing is known about the morphological structure of the uterotubal junction in cheetahs which was investigated to enhance our understanding of this region in wild felids.

**Material and Methods**

Seven formalin embalmed cheetah uteri and associated structures were sourced from  nulliparous captive cheetahs. Six cheetahs, one aged 7 years, two aged 10 years, and two aged 10.5 years (Africat, Namibia) were euthanased as part of the end of life management strategy for the sanctuary and not because of ill health. One cheetah, aged 3 years (De Wildt Cheetah and Wildlife Centre, North West province, South Africa), was presented for post-mortem having died from cryptococcal meningitis. The six Namibian cheetahs were all obtained as individual orphaned wild cheetah cubs less than 1 year-old. The South African cheetah was captive born. The six Namibian cheetahs were housed together and moved annually between fenced camps ranging from 3-50 hectares. The captive born South African cheetah was housed individually in a 1 hectare fenced camp. None of the cheetahs were housed with males nor mated during their lifetime, although male cheetahs were present in adjacent camps. The six Namibian cheetah reproductive tracts were removed during in field post-mortem, performed shortly after death by a private veterinarian. The South African cheetah was presented for post-mortem at the Onderstepoort Veterinary Faculty. The project was approved by the National Zoological Gardens of South Africa's Research and Ethics Committee (Project no. P11/07). A research/collecting permit (1846/2013) was obtained from the Namibian Ministry of Environment and Tourism and the samples were imported into South Africa with the required CITES export (no.0042838) and import (no. 137670) permits, as well as a veterinary import permit (no. 13/1/1/30/2/10/6-2013/11/002397). Once in South Africa, the samples were transported and stored with the required national Threatened or Protected Species (TOPS) ordinary permit (no. 05238).  The tip of each uterine horn, where the UTJ was located, was removed. The left uterine horn tips were sectioned transversely. The right uterine horn tips were sectioned

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longitudinally in 3 cheetahs, and coronally / obliquely in 4 cheetahs, attempting to split the uterine lumen along its length. The coronal sections were obtained by sectioning the uterine tube starting at the extramural part of the isthmus and ending at the uterine ostium. All sections were routinely prepared for histology. Sections were stained with haematoxylin and eosin (H & E), and examined and photographed using an Olympus BX63 light microscope (Olympus corporation, Japan) with bright field illumination. Images were captured using an Olympus DP72 camera (Olympus corporation, Japan). The CellSens Dimension software package (Olympus corporation, Japan) was used to adjust sharpening, contrast and brightness as needed. The Photoshop software package (Adobe, USA) was used to further adjust white balance, merge photomicrographs and make other minor corrections as needed.

## Results

In the cheetah the terminal segment of the isthmus of the uterine tube was situated in the mesosalpinx and positioned just dorsal to the proper ligament. It consisted of a convoluted extramural portion and a straight intramural uterine part (*Pars uterina*) that opened into the tip of the uterine horn via a small papilla which could not be appreciated macroscopically. The terminal isthmus was accompanied by a small blood vessel (Figs. 1 and 2).
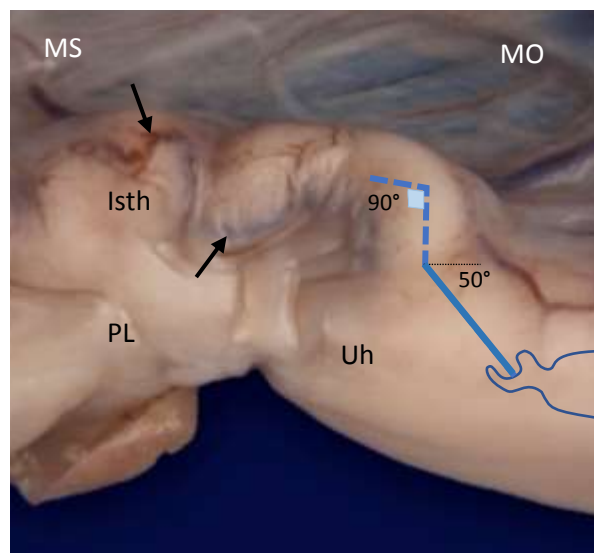


**Figure 1:** Lateral view of the terminal isthmus and uterotubal junction. Cranial is left. The convoluted isthmus (Isth), accompanied by conspicuous blood vessels (arrows), makes a characteristic 90° bend to enter the tip of the uterine horn from where it proceeds caudally through the myometrium as the *Pars uterina* at an angle of approximately 50° to open on a microscopic papilla. The blue overlay demonstrates the lumen of the terminal isthmus (dashed) and *Pars uterina* (thick solid), the papilla and uterine luminal margin (thin solid). Cranial tip of the uterine horn (Uh), Proper ligament (PL), Mesometrium (MO); Mesosalpinx (MS). Bar = 2mm.
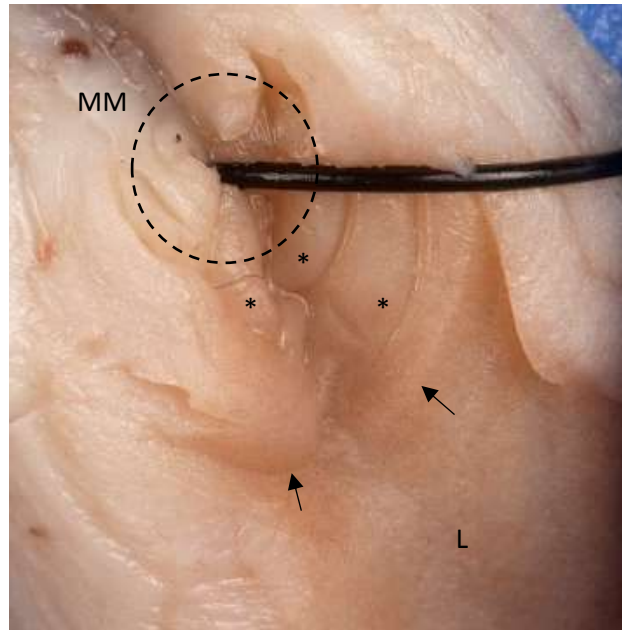
**Figure 2:** Enlargement of the uterotubal junction opened longitudinally from the antimesenterial aspect. Cranial is top left. The uterine lumen (L) has been opened to its apex and the incision continued cranially into the myometrium (MM) to demonstrate the papillary region (black dotted circle). Rounded endometrial ridges (*) surround the uterine ostium and extend a short distance caudally before ending (black arrows). A piece of 3/0 black nylon indicates the position of the uterine ostium. Length of the black nylon = 6mm.

The isthmus penetrated the mesometrial surface of the uterine horn at a point a few millimetres distal to the tip of the horn. It consistently made a 90° bend 2-3 mm prior to entering the myometrium, from where it immediately turned caudally to run at an angle of approximately 50° to the horizontal to open into the termination of the uterine lumen from a microscopic papilla (Fig. 3). The inner circular and outer longitudinal smooth muscle layers constituting the *Tunica muscularis* of the terminal isthmus merged with their uterine counterparts (Fig. 4). The muscle layers were well developed. Approximately 4-5 low, prominent, primary mucosal folds lined by a simple cuboidal to low cuboidal epithelium were present in the uterine part of the isthmus (Fig. 5). The uterine ostium (*Ostium uterinum tubae*) was housed in a dome shaped papilla which protruded from the roof into the terminal part of the uterine lumen. This resulted in the formation of a vault like recess (fornix) between the papilla and walls of the uterine horn (Figs. 3, 4, 6). The papilla consisted of a core of dense irregular connective tissue housing the papillary glands (see below), supported by an extension of the inner circular smooth muscle layer of the isthmus (Figs. 3, 4, 6). Branched tubulo-alveolar glands, morphologically distinct from the uterine glands and referred to here as papillary glands, were a prominent feature of the papilla. The lumen of the papillary glands opened into the uterine lumen (Figs. 3, 4, 6, 7, ).
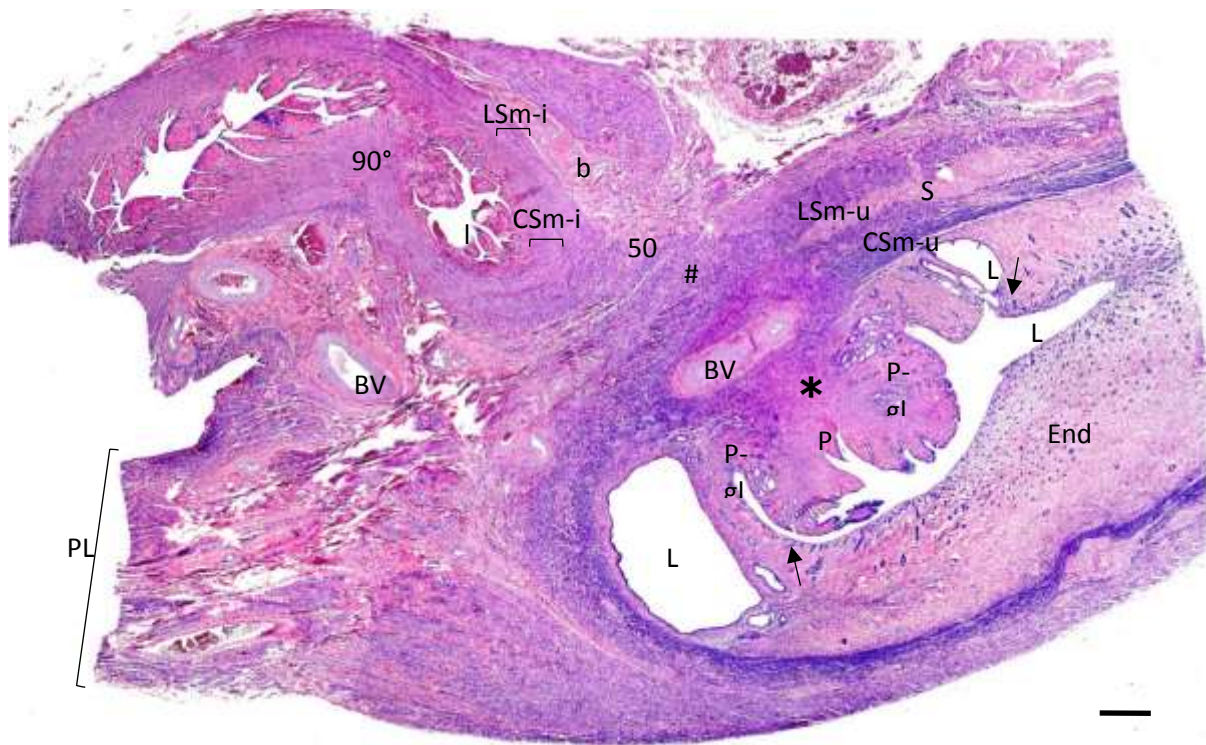
**Figure 3:** Longitudinal section through the UTJ showing the relationship between the terminal parts of the isthmus and the tip of the uterine horn. The isthmus, with its 90° and 50° bends, opens into the uterine lumen (L) via a papilla (P). Note that the papilla is situated a few millimetres caudal to the external junction of the isthmus with the uterine horn. The smooth muscle of the proper ligament (PL) and longitudinal smooth muscle layer of the isthmus (LSm-i) merge with the longitudinal smooth muscle layer of the uterus (LSm-u) at point #. The circular smooth muscle of the isthmus (CSm-i) merges with the circular smooth muscle of the uterus (CSm-u) and continues into the base of the papilla (*) A prominent blood vessel (BV) accompanies the uterine tube and is continuous with the *Stratum vasculare* (SV) of the uterus. The appearance of the papillary glands (P-gl) clearly differs from that of the endometrial glands. Isthmus lumen (l); Endometrium (End). Bar = 600 µm.
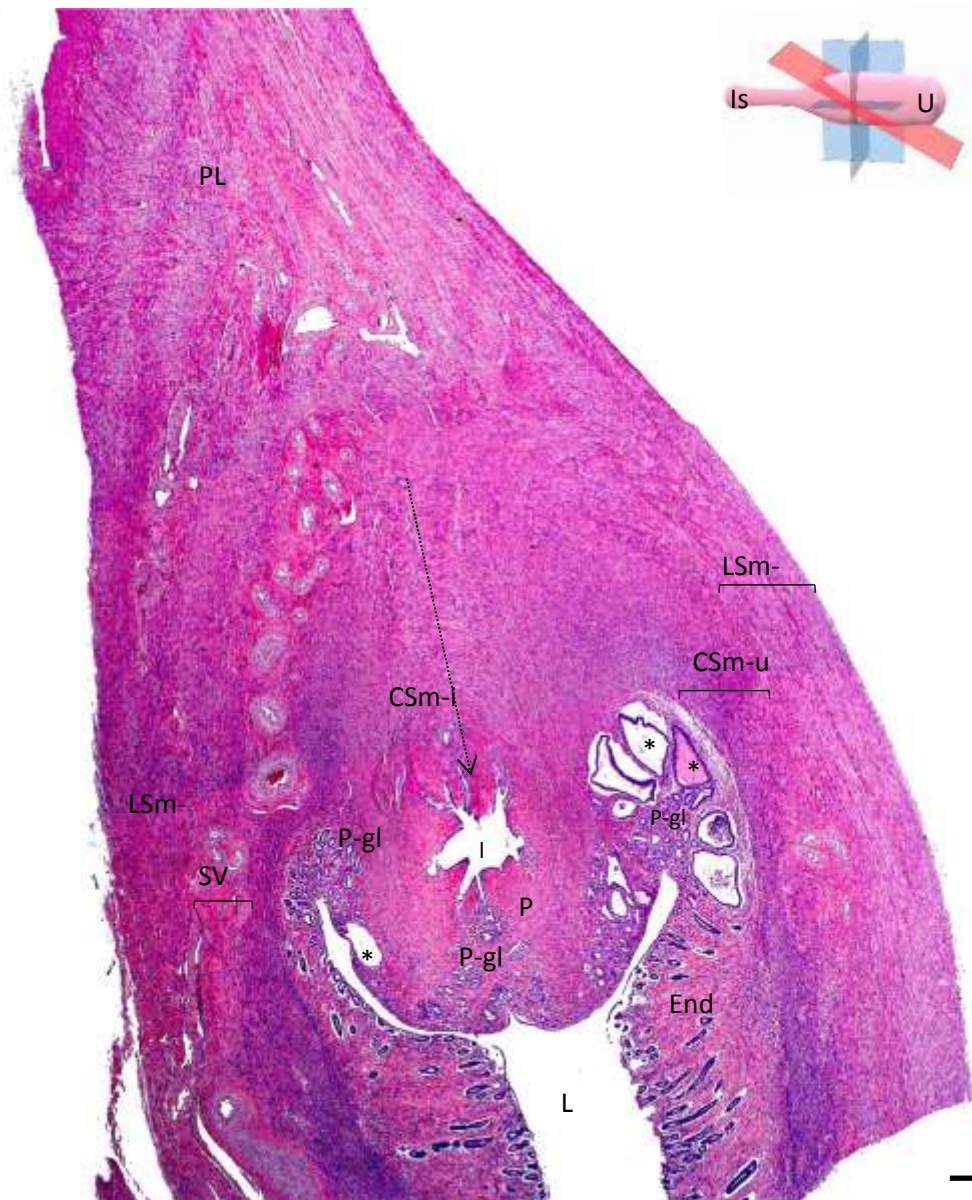
**Figure 4:** An oblique coronal section through the junction of the proper ligament (PL) and isthmus with the tip of the uterine horn. By following the circular smooth muscle layer of the isthmus (CSm-i) the *Pars uterina* can be seen traversing the myometrium in a straight line (black dotted arrow) towards the apex of the papilla (P). The longitudinal smooth muscle of the proper ligament, isthmus and uterine horn blend with each other cranially. The inner circular smooth muscle layer of the isthmus merges with that of the uterus (CSm-u). The papillary glands (P-gl) in the fornix of the papilla are grossly dilated in places (*) and contraction of the epithelium is evident. A pink proteinaceous fluid is present in the lumen of one of the glands with traces in the other adjacent dilated glands. The endometrium (End) thins in the region of the fornix. *Stratum vasculare* (SV); Lumen of the isthmus (l); Uterine lumen (L); Longitudinal smooth muscle layer of the uterine horn (LSm-u). The coloured inset illustrates the plane of section in red (Isthmus (Is); Uterus (U)). Bar = 400 µm.
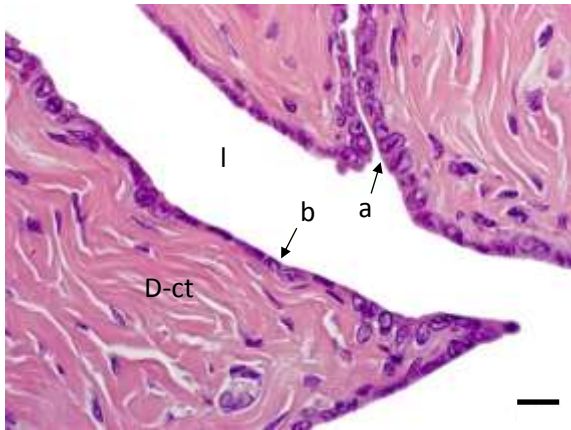
**Figure 5:** Uterine part (*Pars uterina*) of the isthmus. The mucosal folds were lined by a simple cuboidal (a) to low cuboidal epithelium (b). Isthmus lumen (l); Dense irregular connective tissue (D-ct). Bar = 10 μm.

**Figure 6:** An oblique coronal section through the intramural isthmus highlighting the dome shaped papilla (P) and its associated branched tubulo-alveolar papillary glands (P-gl). The *Lamina propria* of the endometrium (End), circular smooth muscle of the isthmus (CSm-i) and papillary glands all contribute to the composition of the papilla. Major blood vessels (BV) are present on either side of the isthmus between the circular and longitudinal (LSm-i) smooth muscle layers. The black arrows mark the appearance of the first uterine glands. The endometrium thins in the region of the fornix. Isthmus lumen (I); Uterine lumen (L); *Stratum vasculare* (SV); Circular smooth muscle of the uterus (CSm-u); Longitudinal smooth muscle of the uterus (LSm-u). The black square is enlarged in Figure 7 &**Error! Reference source not found.**. The coloured inset illustrates the plane of section in red (Isthmus (Is); Uterus (U)). Bar = 400 µm.
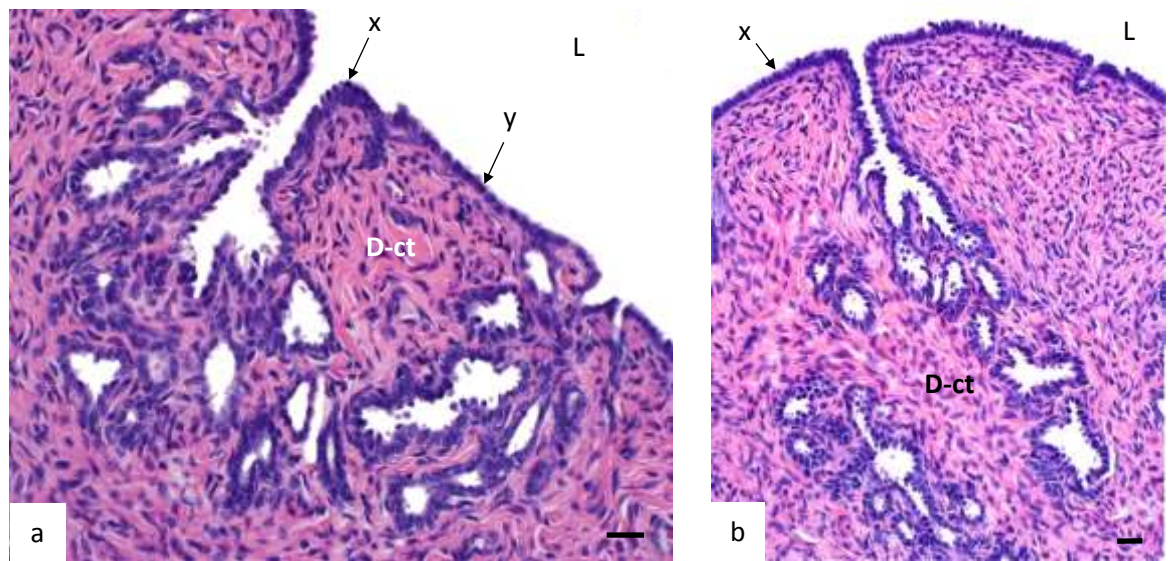
**Figure 7:** Enlargement of the area delineated by the black rectangle in Figure 6. Branched tubulo-alveolar papillary glands demonstrating a squat (a) or elongated (b) profile. Dense connective tissue (D-ct); Uterine lumen (L); simple columnar epithelium (x); simple cuboidal epithelium (y). Bar = 40 μm (a) & 60 μm (b).

The uterine ostium was situated at the apex of the papilla. At the uterine ostium the simple cuboidal or low cuboidal epithelium lining the uterine part of the isthmus abruptly transitioned to a simple columnar epithelium that covered the apex of the papilla. The outer surface of the papilla was generally smooth and symmetrical although some folding of the mucosa was observed in the vicinity of the uterine ostium. Variation in this regard was evident between cheetahs. Approximately halfway from the apex to the base of the papilla the columnar epithelium transitioned abruptly to a simple cuboidal or low cuboidal epithelium consistent with the rest of the uterine lumen. Thus the columnar cells formed a sharply demarcated zone encircling the uterine ostium and covering the apex of the papilla. The stroma of the papilla housing the papillary glands was continuous with that of the endometrium (Figs. 3, 4, 6).

The papillary glands were branched tubulo-alveolar in nature and were found throughout the papilla, from the apex to the base of the fornix. Some glands presented a squat profile (Fig. 7a) while others were long and narrow in appearance (Fig. 7b). They were restricted to the connective tissue component of the papilla and opened into the uterine lumen. The epithelial lining of the glands varied from simple cuboidal to simple columnar, often reflecting the particular surface epithelium from which they were derived. In some sections the glands appeared to concentrate at the base of the fornix. The point of transition between the papillary glands and uterine glands differed between

cheetahs. In some animals they were restricted to the base of the fornix while in others they extended as short distance beyond the base into the endometrium of the uterus ~~(Figs. 3, 4, 6)~~. The interface between the two types of glands was not always clearly defined. The endometrium ~~lining~~ of the fornix was distinctly narrower than that of the uterine horn (Figs. 4, 6). In one animal the papillary glands at the base of the fornix were grossly dilated forming cyst-like structures, some of which were filled with proteinaceous fluid (Fig. 4), sloughed epithelial cells and degenerate neutrophils. The endometrium of the uterus was lined by a simple low cuboidal epithelium (Fig. 8). Low, primarily transverse folds of endometrium, which extended a short distance from the apex of the uterine horn caudally, encircled the papilla constricting the uterine lumen in this area (Fig. 2).
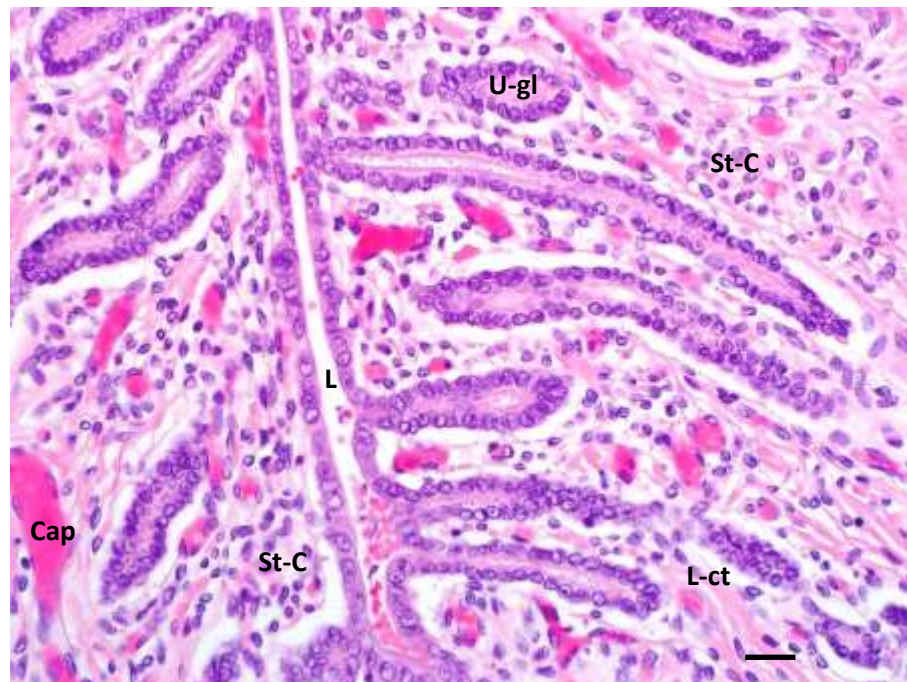


**Figure 8:** The lumen (L) at the tip of the uterine horn is lined by a simple low cuboidal to cuboidal epithelium as are the simple tubular uterine glands (U-gl). Note the numerous capillaries (Cap) and stromal cells (St-C) within the loosely arranged connective tissue (L-ct). Red blood cells in the uterine lumen represent a sampling artefact. Bar = 40 μm.

**Discussion**

The utero-tubal junction has been defined as the region spanning the caudal isthmus to the rostral end of the uterine horn, and may include the entire intramural portion of the caudal isthmus (Hafez & Black, 1969a). The UTJ morphology has been described in various species including the dog, rabbit, rat, sheep, pig, cow, pig-tailed monkey, rhesus monkey (Hook & Hafez, 1968) and horse (Mouguelar *et al.*, 2015). Although the basic structural features of the uterine tube of the domestic cat and lioness have also been addressed, a detailed description of the UTJ is lacking (Crouch, 1969; Bansal & Varinder,

2008; Hartman *et al.*, 2012). No information is currently available on the morphology of the UTJ of the cheetah.

### *Macroscopic features*

Macroscopically, the isthmus of the cheetah generally resembles that of most other domestic animals and the lioness, being fairly tortuous, entering the mesometrial surface of the uterus and opening into the apex of the uterine horn (Dyce *et al.*, 2010; Hartman *et al.*, 2012). In the cheetah the isthmus consistently makes a characteristic 90° bend 2-3 mm prior to entering the myometrium. It then immediately turns caudally at the external myometrial junction to traverse the uterine wall at 50° to the horizontal (130°) to open in the tip of the uterine lumen from a microscopic papilla. In the domestic cat the isthmus enters the uterus at an angle of 120° prior to opening from a papilla, while in the mouse it enters the uterus at 90° and then opens through a mound like protrusion (Hafez & Black, 1969b).

The cheetah is similar to the cat, dog, rat, pig-tailed monkey and rhesus monkey in possessing an intra-mural portion (*Pars uterina*) of the isthmus (Hook & Hafez, 1968; Hafez & Black, 1969b) and which is reportedly absent in the cow, sheep, rabbit and pig (Hook & Hafez, 1968). No specific information on the occurrence of this segment of the isthmus has been presented for the lioness (Hartman *et al.*, 2012 ). An additional feature in the cheetah was the presence of a conspicuous blood vessel on the surface of the isthmus and ampulla not previously reported in other species. As shared with the pig, dog and cat, the primary folds of the isthmus of the cheetah broadened and flattened in the intrauterine part of the isthmus (Hook & Hafez, 1968; Bansal & Varinder, 2008). Flattening of the mucosal folds has also been reported in the sheep (Yániz *et al.*, 2014). The diverticula associated with blind ending crypts described in the terminal isthmus abutting the uterine ostium in the cow (Wrobel *et al.*, 1993) and also reported in the rat, dog, rabbit, sheep, cow, pig, pig-tailed monkey and rhesus monkey (Hook & Hafez, 1968) were not observed in the cheetah.

### *Microscopy of the isthmus*

No major differences were observed between the younger (a 3 year-old and 7 year-old) and older (five 10-10.5 year-old) cheetahs, or between cheetahs of the same age. Occasional minor individual differences are noted under the relevant sections below.

The luminal epithelium of the *Pars uterina* in the cheetah was similar to that in the dog and rat, being composed exclusively of non-ciliated cells (Hook & Hafez, 1968). In contrast, species such as the cow,

sheep, rabbit and pig display an epithelium consisting of both ciliated and non-ciliated cells (Hook & Hafez, 1968). To what extent this difference in cell types reflects the existence of the *Pars uterina* in some species remains unknown. The nature of the luminal epithelium of the *Pars uterina* in the cat has not been reported (Bansal & Varinder, 2008).

The *Lamina propria* of the cheetah resembled that of the dog, rat, rabbit, cow, sheep, pig, pig-tailed monkey and rhesus monkey in consisting predominantly of dense irregular connective tissue positioned between the luminal epithelium and the *Tunica muscularis* (Hook & Hafez, 1968). As reported in other species (Hook & Hafez, 1968) its outer aspect abutting the *Tunica muscularis* was evenly contoured while the inner aspect formed the bulk of the mucosal folds of the uterine tube.

In the human (Rocca *et al*., 1989), bovine (Wrobel *et al*., 1993), rat, sheep and rabbit (Hook & Hafez, 1968) the *Tunica muscularis* of the intramural part of the isthmus displays an additional inner longitudinal muscle layer. This layer is absent in the cheetah similar to the situation in the pig, dog (Hook & Hafez, 1968) and cat (Bansal & Varinder, 2008; Hafez & Black, 1969b). Information is lacking for the lioness (Hartman *et al*., 2012).

### The papilla

Species differences have been reported regarding the interface between the uterine tube (isthmus) and uterine horn. In the cheetah the isthmus opened into the uterine horn from a microscopic papilla. This observation represents the first report of a papilla housing the UTJ in wild felids.  However, this feature is not unique to the cheetah. The uterine tube terminates in a papilla in the cat although details regarding its morphology are lacking (Crouch, 1969; Hafez & Black, 1969b). No mention is made of a macroscopically visible papilla in the lioness (Hartman *et al*., 2012) although the possible presence of a microscopic papilla similar to that observed in the cheetah cannot be ruled out. The uterine tube also opens on a papilla in the horse (Mouguelar *et al*., 2015) and rat, whereas in the dog and mouse the isthmus opens through a slit like ostium on a mound that projects into the uterine lumen (Hook & Hafez, 1968; Hafez & Black, 1969b). In contrast, in the cow, sheep and monkeys the uterine horn simply tapers into the uterine tube (Hook & Hafez, 1968).

Species variations in surface topography have been noted at the interface between the uterine tube and the uterus. Oviductal projections are present at the ostium of the uterine tube in the pig and the rabbit. The endometrial stroma of the uterus adjacent to the uterine ostium forms a circular fold around these projections in the rabbit. In the pig, folds of the endometrium supplied with uterine glands are found below the oviductal projections (Hook & Hafez, 1968) while in the cheetah a series

of low endometrial folds encircle the papilla. While their function remains speculative, they may play a role in regulating sperm access to the uterine tube (Hunter, 2002).

### *Unique morphological properties of the papilla*
### *The epithelial lining*

The papilla housing the UTJ in the cheetah exhibited interesting structural peculiarities. In respect of the epithelial lining a transition zone could be discerned, marked by an abrupt change from a simple cuboidal or low cuboidal epithelium lining the isthmus, to a simple columnar epithelium at the uterine ostium. This columnar epithelium extended approximately half way to the base of the papilla before abruptly changing to reflect the normal simple cuboidal to low cuboidal uterine epithelium. In the domestic cat, the epithelial lining of the uterine tube is a simple mixed columnar and ciliated columnar epithelium demonstrating a progressive decrease in columnar cell height from the infundibulum to the isthmus, with the columnar epithelium of the isthmus being half the height of the infundibular epithelium. However, the epithelial transition at the UTJ has not been described (Bansal & Varinder, 2008). A transitional area is also apparent in humans. Here the simple cuboidal epithelium present in the vicinity of the uterine ostium changes to the simple columnar epithelium of the endometrium together with the appearance of uterine glands (Rocca *et al*., 1989). Similarly, uterine glands make their appearance in the transition zone in the bovine (Wrobel *et al*., 1993). In sheep there is a progressive transition from the predominantly ciliated isthmus epithelium to the less ciliated uterine epithelium, again associated with the appearance of the uterine glands (Yániz *et al*., 2014).

The stage of the estrus cycle should be considered when describing or comparing the surface epithelium of the UTJ as this may influence cell morphology. In the cow, for example, both ciliated and non-ciliated cells are present in the UTJ with ciliation increasing during proestrus and estrus (Wrobel *et al*., 1993). In contrast, ciliation remains unchanged during the reproductive cycle in the isthmus of the Thai Swamp buffalo (Tienthai *et al*., 2009) and sheep (Yániz *et al*., 2014). The interface between the ciliated cells in the transition zone in the cow and the sparsely ciliated surface epithelium of the uterus shifts in a uterine direction during proestrus and estrus and retreats during interestrus and diestrus. Additionally, this boundary is not clear cut and runs an intricate course with interdigitating projections emanating from either side of the interface (Wrobel *et al*., 1993). In the cat, ciliation of the uterine tube epithelium is mediated by the presence of estrogen and is highest during estrus. In the presence of progesterone, during the post ovulation phase after mating or during pregnancy, many of the epithelial cells deciliate in the uterine tube and almost halve in height. Many of these cells also undergo apoptosis (Verhage *et al*., 1984). Whether similar cyclical changes occur in the cheetah

remains undetermined. However, although the stage of the reproductive cycle in the cheetahs examined is unknown, it is most likely to be anestrous. The cheetah is mostly an induced ovulator that undergoes waves of follicular development with associated estrogen concentrations in the body and receptive behaviour (Brown *et al.*, 1996). If not mated, the cohort of developing follicles regresses and the cycle repeats (Andrews *et al.*, 2018). These cycles occur year round with estrogen peaks occurring on average every 9.9 +- 2.2 days, with older females displaying shorter intervals (Crosier *et al.,* 2017). However, these active, short bursts of estrogenic activity are interrupted in most captive cheetah by significant periods of ovarian acyclicity, the majority of which occur during winter or spring (Crosier *et al.,* 2017). The reproductive tracts of the 7 cheetahs examined contained no evidence of pregnancy nor recent ovulations. No *Corpora lutea* were observed on the ovaries. All cheetah ovaries showed signs of ovarian activity with secondary follicles and tertiary follicles being observed. However, the ovaries were small, ranging from 260.32 mm$^3$ – 1091.08 mm$^3$ (calculated using the method of Pavlik *et al.* (Pavlik *et al.*, 2000 ) and a formalin shrinkage correction factor of 1.22)(Partin *et al*., 1989), with the largest follicle measured on each ovary ranging from 1-1.7 mm in diameter consistent with anestrous ovaries and immature follicles respectively (Schulman *et al.,* 2015). A 2.5 mm follicle was observed in the 7 year-old cheetah. Uterine sections showed no signs of oedema and the uterine glands were straight and sparse, with no secretory activity evident, and lined by a cuboidal to low cuboidal epithelium. All 7 cheetahs showed a similar ovarian size, follicle size and uterine gland picture consistent with a low estrogen state most likely associated with anestrous (Schulman *et al.,* 2015). The chance of this synchronised low estrogen picture occurring amongst the 6 group housed cheetahs is unlikely if they were actively cycling as it would be expected that some cheetahs would be in proestrus and estrus, with associated larger follicles (4-6mm) (Schulman *et al.*, 2015) and signs of estrogenisation in the uterus. Additionally, the South African cheetah died during autumn and the Namibian cheetahs were euthansed mid-winter when acyclicity is most prevalent (Crosier *et al.,* 2017). Based on all of the above factors, we conclude that the cheetahs are more likely to be in anestrous than interestrus. Levels of progesterone and estrogen, as well as vaginal cytology(Schulman *et al.,* 2015), were not available to help confirm this assumption. It therefore cannot be ruled out that the simple low cuboidal to cuboidal epithelium of the intrauterine part of the isthmus might double in height and even develop cilia during periods of higher estrogenisation such as during estrus.

### *The papillary core*

The core of the papilla in the cheetah contained a substantial amount of smooth muscle emanating from the inner circular smooth muscle layer of the *Tunica muscularis* of the isthmus and which blended with, and projected through, the corresponding circular smooth muscle layer of the uterus into the

papilla. This corresponds with findings in the cat (Hafez & Black, 1969b). In the rat, the papilla is predominantly composed of the endometrial lamina propria with circular smooth muscle present in its base (Hook & Hafez, 1968). The endometrial stroma of the uterus adjacent to the uterine ostium forms most of the core of the mound in the dog (Hook & Hafez, 1968). The absence of uterine glands in the papilla of the cheetah has also been observed in the papilla of the rat, information in this regard is lacking for the mound of the dog (Hook & Hafez, 1968). However, unique to the papilla in the cheetah is the presence of branched tubulo-alveolar glands and a zone of epithelial cells surrounding the uterine ostium that is morphologically distinct from the lining of the isthmus and uterus.

The branched tubulo-alveolar glands demonstrated in the papilla in the cheetah were morphologically distinct from the uterine glands and represented a unique finding. In the bovine, short tubulo-alveolar crypts have been described in the transition zone associated with the depth of the diverticula in the terminal isthmus and identified as distinct from the uterine glands (Wrobel *et al.*, 1993). In equines, crypts resembling glands have been noted throughout the uterine tube (Mouguelar *et al.*, 2015). However, as far as could be ascertained, this is the first time that branched tubulo-alveolar glands have been described in the UTJ of any species. Dilation of the papillary glands, most prominent at the junction of the endometrium with the stroma of the papilla, was frequently observed amongst the cheetahs although the extent of the dilation varied and uterine gland structure remained unaffected. The cells lining the dilations showed no obvious signs of pathology. However, the overt dilatation of the papillary glands observed in a 10.5 year-old cheetah (see Fig. 4) may represent a pathological process as degenerate neutrophils and sloughed epithelium was observed in the proteinaceous fluid of the glands. This was not observed in the variably dilated glands of the other cheetahs. No additional staining of the proteinaceous fluid was done, and the exact reason for the overt distention and presence of the proteinaceous fluid remains unknown, but may represent the start of cystic endometrial hyperplasia, a common uterine condition in older cheetahs (Crosier *et al.*, 2011). The dilations of the papillary glands in the cheetahs could easily be confused with this condition if the uterine tip was submitted for histopathology and was sectioned through the region of the UTJ. Until further research can clarify the association of the glandular dilations with a pathological condition or as normal for the cheetah, it would be wise for samples submitted for histopathology of the uterus to be taken at least a few millimetres caudal to the UTJ. Since this point is not macroscopically visible it would be advisable to sample at least 5 mm caudal to the external junction of the isthmus with the uterine horn. No literature could be sourced on dilation of UTJ glands; this is not surprising as very little literature exists on the presence of UTJ glands in other species (Mouguelar *et al.*, 2015; Wrobel *et al.*, 1993).

### Significance of the smooth muscle and glands

The significance of the glandular and muscular nature of the papilla in the cheetah is unknown, although the papilla presumably plays a role in regulating the passage of sperm into the uterine tube as described in other species (Chatdarong *et al*., 2004; Holt & Fazeli, 2016). The muscular walls of the isthmus have been shown to block the movement of ova into the uterus for a number of hours post ovulation in bovines (Black & Davis, 1962). The thick muscular layer of the isthmus and the papilla suggests a similar mechanism may exist in the cheetah. This would presumably ensure that ova are fertilised in the uterine tube in the cheetah as in the domestic cat (Knospe, 2002). The well-developed and morphologically distinct papillary glands observed in the cheetah may function as a reservoir for the storage of glandular secretions or act as a sperm storage area prior to ovulation. This would be consistent with a report in the domestic cat where the UTJ and uterine glands act as preovulatory sperm storage areas (Chatdarong *et al*., 2004). The need for a sperm reservoir is unavoidable in the cheetah as they are mostly induced ovulators (Brown *et al.*, 1996) and sperm needs to be stored until ovulation has occurred. Recent research into sperm reservoirs reveal that complex mechanisms exist to regulate the passage of sperm into the uterine tube and protect sperm viability (Holt & Fazeli, 2016; Suarez, 2016). Both mechanical and molecular mechanisms play an important role (Suarez, 2016). Sperm represent a foreign material in the body of the female and stimulate an immune response that helps clear them from the female reproductive tract (England *et al.*, 2013b; Holt & Fazeli, 2016). A balance must be met between clearing foreign material, including pathogens, from the reproductive tract while allowing sperm to survive long enough in a sperm reservoir to effect fertilisation (England *et al.*, 2013a; Suarez, 2016; Atikuzzaman *et al.*, 2017). After entering the uterus, sperm congregate in the tips of the uterine horns around the UTJ where they bind in clumps to the ciliated epithelium in the dog which may allow them to conserve energy while they wait for the ovum to enter the ampulla (England *et al.*, 2013a). Passage through the UTJ is regulated by both the physical constraints of the UTJ opening, resulting in only a privileged population of sperm finding it, and also molecular screening of the sperm (England *et al.*, 2013a; Suarez, 2016). Not all sperm can enter the UTJ (Suarez, 2016). The epithelium of the UTJ seems capable of screening the proteins on the acrosome of the sperm, rejecting some and accepting others (Suarez, 2016). The exact proteins and mechanisms involved in this selection process remain under investigation (Suarez, 2016). The mechanisms of detachment of the resting sperm, timing of sperm release from the sperm reservoir, migration up the uterine tube to reach the ovum in the ampulla, capacitation and fertilisation of the ovum are likely controlled by complex signalling pathways that remain to be unravelled (Suarez, 2016). Sperm progression through the female reproductive tract is also affected by the shape of the reproductive tract (Suarez, 2016). Micro-channels in the cervix of the cow selectively allow sperm to swim against cervical fluid flow

while pathogens are swept away (Mullins & Saacke, 1989). The microstructure of the UTJ of the cow may help orient sperm to swim in the correct direction towards the ovum (Suarez, 2016). Viscosity of reproductive tract fluids also help or hinder sperm progression as shown by the blocking effect of the mucosal plug in pigs (Rodriguez-Martinez *et al.*, 1990). Further research in cheetahs, possibly utilizing scanning electron microscopy of the UTJ and molecular studies of the UTJ epithelium and sperm, would be needed to corroborate findings with other species. Whether this presumed sperm reservoir, or passage of sperm through the UTJ in the cheetah shares molecular similarities to other mammals, as discussed above, remains to be investigated.

This study revealed several interesting morphological features of the UTJ of the cheetah. A sharply demarcated circular zone of columnar epithelial cells was located at the uterine ostium and separated the simple cuboidal to low cuboidal epithelium of the isthmus and uterine epithelium from each other. Previously unreported branched tubulo-alveolar glands were observed in the papilla housing the uterine ostium and may act as sperm storage areas. Dilation of the papillary glands in some cheetahs could easily be confused with cystic endometrial hyperplasia.

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