Distribution of uterine histological changes in aged captive cheetahs (*Acinonyx jubatus*)

Miles J. Penfold[1,*], Martin L. Schulman[2], Sarah Clift[3], Lisa du Plessis[4], Peter N. Thompson[5,6], Marthinus J. Hartman[1]

[1]Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
[2]Section of Reproduction, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
[3]Section of Pathology, Department of Paraclinical Sciences, Faculty of VeterinaryScience, University of Pretoria, Onderstepoort, South Africa
[4]Idexx Laboratories, Centurion, South Africa
[5]Section of Epidemiology, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
[6]Centre for Veterinary Wildlife Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

*Correspondence
Miles J. Penfold, Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa.
Email: miles.penfold@up.ac.za

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Abstract

The histological effect on the felid uterus of sterilization, via ovariectomy or salpingectomy, is currently unknown. To investigate the association of ovariectomy or salpingectomy with uterine health, it is first necessary to establish if changes are distributed evenly throughout the uterus. Both laparoscopic ovariectomy and salpingectomy with concurrent sampling of the tip of the uterine horn are possible in the cheetah. Currently accepted practice for histopathological screening of the uterus utilizes four biopsy samples. It is not known whether this method accurately reflects the status of the entire uterus. In this study we histologically examined the uteri of six older cheetahs (one 7-year-old and five 10–10.5-year-old animals) via 21 tissue samples (three samples from seven different anatomical regions) per cheetah to determine overall uterine health. Although no defined lesions were detected, mild endometrial gland dilation, assumed to be of no functional consequence, was observed in multiple samples. The odds of observing this dilation was lowest in the uterine body and progressively increased in a cranial direction, being significantly higher at the tip of the uterine horns (OR = 11.5; 95% CI, 2.0-65.1; $p = 0.006$). This supported the reliability of sampling the tip of the uterine horn to screen for endometrial gland dilation.

Keywords: biopsy, endometrial gland dilation, felids, reproduction

1 INTRODUCTION

Captive breeding of cheetahs has met with varied success (Brown, 2011; O'Brien et al., 1985). More than 90% of wild female cheetahs have been reported to reproduce (Laurenson, Caro, & Borner, 1992) compared to less than 20% in captivity (Marker-Kraus & Grisham, 1993). Both species-specific factors (O'Brien et al., 1985), including inability to reliably identify females in estrus (Brown, 2011), and poor husbandry practices reportedly contribute to this poor reproductive performance (Caro & Laurenson, 1994). Additionally, sperm counts in male cheetahs are 10 times lower than in male domestic cats and sperm morphology abnormalities average 71% (O'Brien et al., 1985) compared to 49% in male domestic cats during the reproductive season (Axnér & Linde Forsberg, 2007). Furthermore, captive bred cheetah cubs have a high infant mortality rate of 29.1% before reaching 6 months (O'Brien et al., 1985). Ovarian volume, follicle dimension, vaginal cytology and serum progesterone concentrations have been previously reported to establish stage of the reproductive cycle in the cheetah (Schulman et al., 2015). Serum progesterone concentrations and vaginal cytology were not available in this investigation, and consequently other parameters were considered.

The literature on the North American captive cheetah population reports numerous uterine pathologies, including endometrial atrophy, hyperplasia, cysts, polyps and fibrosis, endometritis, chronic lymphocytic endometritis, hydrometra, pyometra and adenomyosis, that become more prevalent with age (Crosier et al., 2011; Munson, Gardner, Mason, Chassy, & Seal, 2002). These conditions are thought to prevent embryo implantation (Wolf, 1992) or early stage nidation (Crosier et al., 2011; Munson et al., 2002). In the Crosier study 50% of prime (6−8 years) and 87.5% of old (9–17 years) cheetahs displayed endometrial hyperplasia (Crosier et al., 2011). Although progestagen usage to control breeding has been associated with uterine pathologies in wild felids (Munson et al., 2002), many captive cheetahs that have never been exposed to progestagens display similar uterine pathologies thought to negatively impact upon fecundity such as cystic endometrial hyperplasia (CEH) (Crosier et al., 2011). Although poor husbandry and management practices have been shown to significantly negatively impact breeding success in captive cheetahs (Brown et al., 1996; Wielebnowskir, Ziegler, Wildt, Lukas, & Brown, 2002), Crosier et al. (2011) recently concluded that the decreased fertility routinely observed in older female cheetahs in captivity was predominantly as a direct result of CEH and other related serious uterine pathologies. They concluded that further research was needed to scrutinize the role of steroidal influences on uterine health in the cheetah (Crosier et al., 2011).

Ultrasound has not proved sufficiently sensitive for clinical diagnosis of CEH or other uterine pathologies at an early stage, thus histology remains the gold standard for diagnosis (Crosier et al., 2011; Schulman et al., 2015). In women, sampling methods include ultrasound guided biopsy, blind endometrial biopsy using a pipelle device, directed biopsy during hysteroscopy, dilatation and curettage, and pathological assessment post hysterectomy (Hannemann, Alexander, Cope, Acheson, & Phillips, 2010). The challenge in the cheetah is the current inability to laparoscopically obtain endometrial samples using biopsy forceps. In addition, in women, mechanical dilation of the cervix followed by scraping of the endometrium can be used to obtain samples (Hannemann et al., 2010). However, mechanical irritation of the endometrium may be associated with CEH as was reported in dogs (De Bosschere, Ducatelle, & Tshamala, 2002). Transcervical biopsy may also predispose to endometritis or pyometra through iatrogenic introduction of bacteria into the uterus (Christensen et al., 2012; Günzel-Apel, Wilke, Aupperle, & Schoon, 2001). Transcervical catheterization has been successfully performed in both the domestic dog, cat (Romagnoli & Lopate, 2014), tiger (Wildt et al., 1987), lion (Pope, 2000), Amur leopard and cheetah (Goeritz et al., 2012). In the African lioness the cervix is intra-pelvic, short (1.4–2.1 cm) and thick walled (1.3–1.6 cm diameter) with fine longitudinal folds (Hartman, Groenewald, & Kirberger, 2012). Although the morphology of the cheetah cervix is unreported, histologically it consists of 4–5 large primary mucosal folds surrounded by a *Tunica muscularis* with no elastic fibers present (Penfold, 2019). Laparoscopically, the tip of the uterine horn in the cheetah has been successfully harvested using a LigaSure™ device (Hartman et al., 2015). To the authors' knowledge, there are no reports in the cheetah describing sampling methods as are described in women (Hannemann et al., 2010). The medium to long-term effects of ovariectomy and salpingectomy on the felid uterus are unknown. The initial step in investigating the effect of these surgical procedures on felid uterine health requires establishing whether biopsies from different regions of the uterus are representative of the entire uterus.

The primary objective of this study was to assess the distribution of uterine histological changes in nulliparous, aged captive cheetahs. Data derived from medical records and reported husbandry practices and the concurrent measurement of ovarian volume and histology in combination with uterine histological findings were used to define the reproductive status of the cheetahs.

2 MATERIALS AND METHODS

2.1 Animals

Post-mortem derived reproductive tracts were sourced from six nulliparous, 7–10.5-year-old (one 7-year-old; two 10-year-old; three 10.5-year-old) captive female cheetahs from AfriCat, Otjiwarongo, Namibia under Research permit NZG/P13/11.

All animals originated as orphaned wild cubs and were maintained together as a group of non-releasable wild cheetahs under free-range conditions in large, fenced, bushveld camps ranging in size from 3 to 50 ha. They were rotated between camps approximately once a year. This necessitated immobilization via darting, which facilitated annual health checks, vaccination, deworming and other measurements. No males were enclosed with the females, although males were present in groups in adjacent camps separated by a fence line or fenced roadway. No contraceptive treatments were administered to the female cheetahs at any stage. The cheetahs were fed a ration of Iams™ kitten and Iams™ adult chicken cat food (Iams™, Mason) twice a week and donkey or horse meat on the bone three times a week, obtained from local abattoirs or farmers and fortified with a commercial vitamin and mineral supplement (Predator Supplement; V-Tech, Centurion, South Africa). The cheetahs were sedated with medetomidine (Domitor®, Zoetis South Africa (Pty) Ltd., Sandton, South Africa) and ketamine (Anaket V®, Bayer (Pty) Ltd., Kempton Park, South Africa) and then euthanased with sodium pentobarbitone intravenously (Eutha-naze®, Bayer (Pty) Ltd., Kempton Park, South Africa) as a part of the sanctuary's end-of-life program in June 2012.

The reproductive tracts were examined macroscopically and gross pathological changes were recorded. As blood samples were not collected at post-mortem, the serum ovarian steroid levels are unknown.

A Section 20 Permit (no. 12/11/1/1/18) to perform research was obtained from the Department of Agriculture, Forestry and Fisheries. The project was approved by the National Zoological Gardens of South Africa's Research and Ethics Committee (project no. P13/11) and the University of Pretoria Research and Ethics Committee (project no. V089-17). 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 transferred and stored with the required national Threatened or Protected Species (TOPS) ordinary permit (no. 05238).

2.2 Categorization of cyclic activity

Based on a combination of follicle sizes, ovarian volumes, endometrial gland morphology and density, and other uterine histological changes, the cheetahs were categorized as being cyclically active (proestrus, estrus, diestrus) or inactive (anestrus) (Davoudi, Zavareh, Ghorbanian, & Hassanzadeh, 2015; Schulman et al., 2015).

2.3 Ovarian structures and volume

Ovarian dimensions were measured using a metric ruler to calculate the ovarian volume (Pavlik et al., 2000). A correction factor of 1.22 (Partin, Epstein, Cho, Gittelsohn, & Walsh, 1989) was subsequently applied to compensate for shrinkage due to 6 years of formalin storage. Ovaries were subdivided evenly into five transverse sections. The right ovary of Cheetahs 3 and 4, and the left ovary of Cheetah 1 were sectioned sagittaly into two parts to facilitate follicle visualization in these sagittaly flattened ovaries. The sections were routinely processed (Wolfe, 2019), embedded in paraffin and stained with hematoxylin and eosin. Ovarian sections were examined for functional structures (*Corpora lutea* and follicles) and pathologies or anomalies including granulosa cell tumors, paraovarian cysts (POCs), Wolffian duct remnants, *Rete ovarii* cysts, neoplasia, mineralization, salpingitis and hydrosalpinx. Follicle diameters were recorded by measuring both the widest diameter and the corresponding perpendicular diameter taken from the midpoint of the first measurement. The average of these two values was used to determine a follicle diameter (Griffin, Emery, Huang, Peterson, & Carrell, 2006).

2.4 Uterus

The uterus was sampled transversely in the cranial, middle and caudal thirds of the length of each horn and at the midpoint of the uterine body (Figure 1). Three transverse, 3-mm-thick samples obtained from each region were placed in a nonmirrored orientation within separate tissue sample cassettes. The 1-mm section between each sample was discarded to prevent mirroring. The cranial third of the horn was sampled starting 5 mm caudal to the junction of the uterine horn with the uterine tube and extending caudally so as to avoid the uterotubal junction and its associated papillary glands (Penfold, Soley, & Hartman, 2019). The sections were routinely processed (Wolfe, 2019), embedded in paraffin and stained with hematoxylin and eosin. The resultant 4 µm sections were examined sequentially at ×10, ×40, and ×100 magnification for observation of a list of previously reported changes comprising mineralization, edema, hyalinosis, neoplasia, pyometra, hydrometra, endometritis (suppurative or lymphoplasmacytic), hyperplasia (cystic, papillary, or adenomatous), adenomyosis and leukocytosis (Crosier et al., 2011; Munson et al., 2002). Endometrial glands were evaluated for density, degree of coiling, dilation, nesting, glandular content and papillary projections. All three examiners were blinded to the identity of the sections being examined. Representative images of the endometrium were captured using an Olympus® DP72 camera (Olympus Corporation, Tokyo, Japan) mounted on an Olympus® BX63 light microscope (Olympus Corporation, Tokyo, Japan) with bright field illumination. The Olympus® CellSens Dimension® software package (Olympus Corporation, Tokyo, Japan) adjusted for sharpening, contrast and brightness as needed. The Adobe® Photoshop software package (Adobe®, San Jose, United States of America) further adjusted white balance and made other minor corrections as needed.

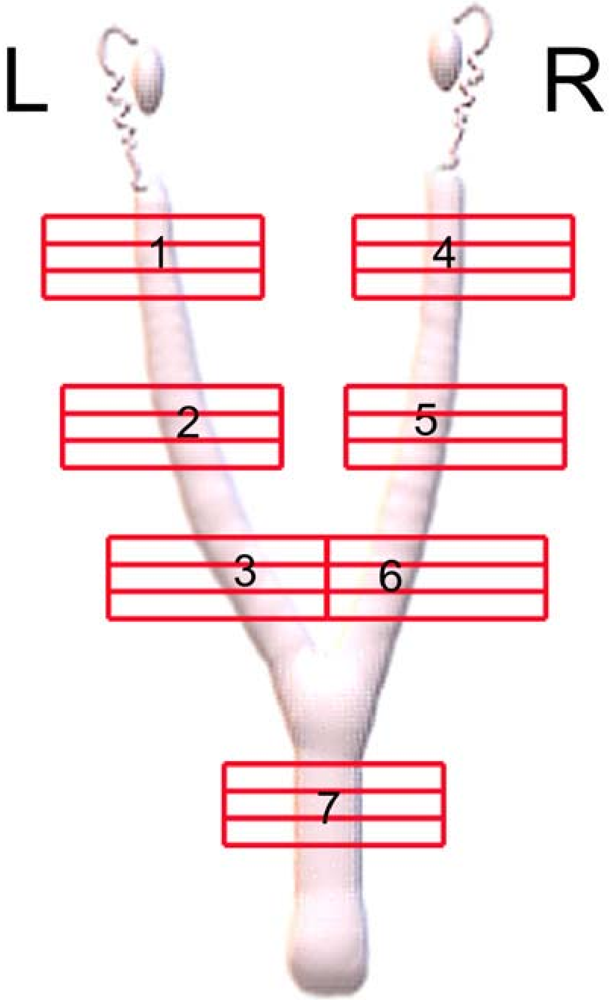


Figure 1. Schematic diagram of the cheetah uterus. Red rectangles represent the three 3-mm-thick samples obtained post-mortem from each uterine regions 1–7

2.5 Endometrial thickness

Endometrial thickness was measured using the CellSens Olympus® Dimension software package (Olympus Corporation, Tokyo, Japan). The thickest and thinnest regions of each sample were measured and an average of the two measurements recorded.

2.6 Statistical analysis

Left and right ovarian volumes were compared using the Wilcoxon matched pairs signed-ranks test. Normality of endometrial thickness data was assessed using the Shapiro-Wilk test; measurements were then log-transformed and compared between sites and sides using a general linear mixed model with random effect for animal. As only mild endometrial gland dilation was observed, the relative severity was not assessed and subsequent analysis considered only presence or absence rather than degree of severity. The association of site (horn tip; horn body; horn base; uterine body) and side of horn (left or right) with the presence of dilation in the sections was assessed using a general linear mixed model with binomial outcome distribution and logit link function, and a random effect for animal. Analysis was done using Stata® 15 statistical software (StataCorp LLC, College Station, United States of America) and statistical significance was assessed at $p < .05$.

3 RESULTS

The clinical records showed that the overall health of all cheetahs was good at their time of death. Two animals showed signs of Grade 3 gastritis and dental attrition at post-mortem, three showed signs of dental attrition alone and one was diagnosed with haemosiderosis of the meninges.

All the uteri examined were obviously nonpregnant and no gross pathology was identified. Histologically the endometrial glands were predominantly straight and sparse (Figure 2a). No signs of estrogenisation (edema; uterine gland proliferation; hyperemia) (Davoudi et al., 2015) were apparent.

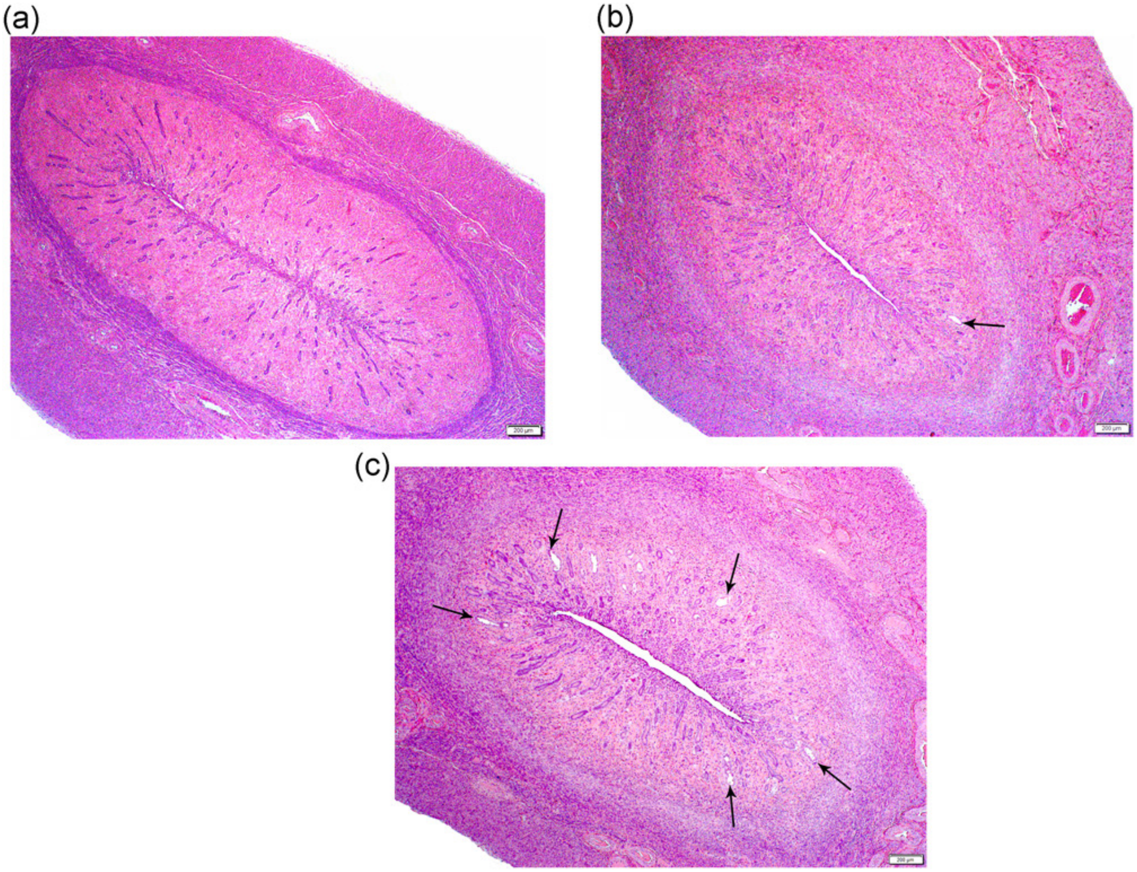
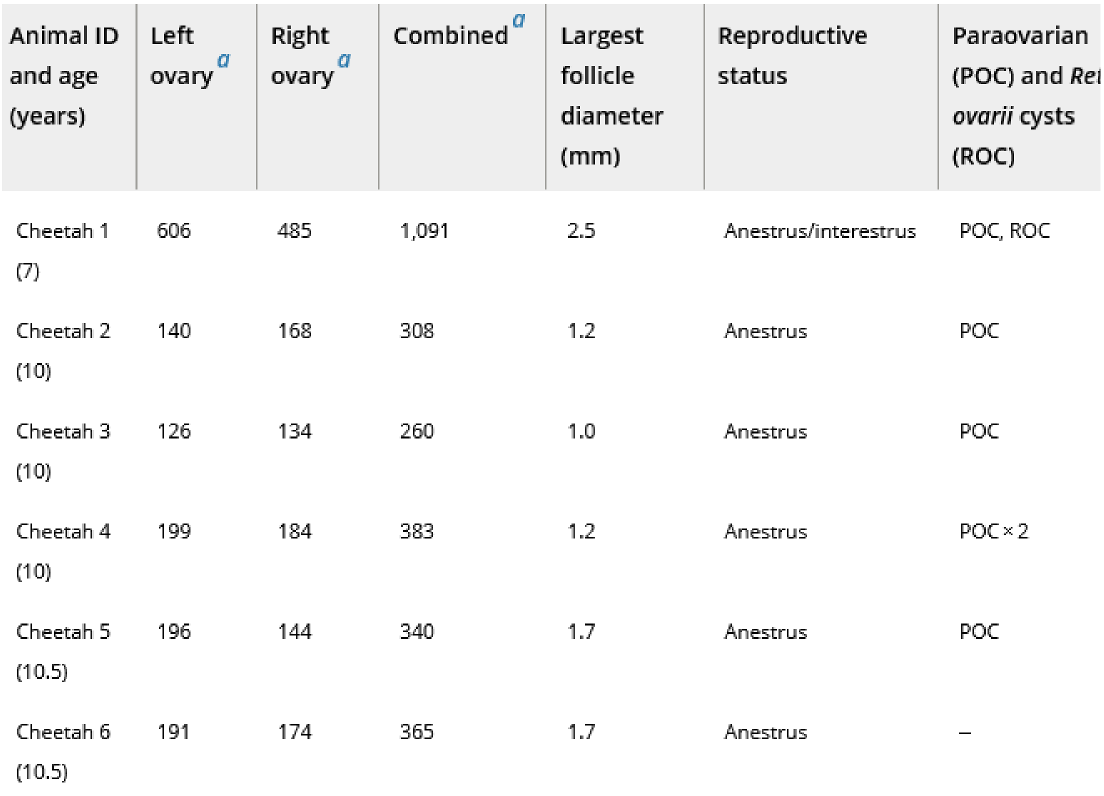


Figure 2. Uterine histology and spectrum of endometrial gland dilation. Endometrial glands are predominantly straight and sparse. Endometrial gland dilation (arrows) varied from: absent (a); single gland affected (b); and multiple glands affected (c)

3.1 Categorization of cyclic activity

Based on the combination of follicle size, ovarian volume, endometrial gland morphology and density, and other uterine histological changes, 5/6 (83%) cheetahs were categorized as anestrus and 1/6 (17%) as anestrus or interestrus (Table 1).

Table 1. Ovarian dimensions, associated structures and categorization by reproductive status derived post-mortem from six nulliparous female Namibian cheetahs



Animal ID and age (years)	Left ovary [a]	Right ovary [a]	Combined [a]	Largest follicle diameter (mm)	Reproductive status	Paraovarian (POC) and *Ret* *ovarii* cysts (ROC)
Cheetah 1 (7)	606	485	1,091	2.5	Anestrus/interestrus	POC, ROC
Cheetah 2 (10)	140	168	308	1.2	Anestrus	POC
Cheetah 3 (10)	126	134	260	1.0	Anestrus	POC
Cheetah 4 (10)	199	184	383	1.2	Anestrus	POC × 2
Cheetah 5 (10.5)	196	144	340	1.7	Anestrus	POC
Cheetah 6 (10.5)	191	174	365	1.7	Anestrus	–

[a] Ovarian volumes (mm^3) calculated (Pavlik et al., 1989) with correction factor = 1.22 for formalin shrinkage (Partin et al., 1989).

3.2 Ovarian structures and volumes

In six cheetahs there was no difference between left (median, 194 mm^3; interquartile range [IQR], 140–199; range, 126–606) and right (median, 171 mm^3; IQR, 144–184; range, 134-485) ovarian volumes (p = .249). The ovaries of all six animals showed cohorts of follicles at different developmental stages, primordial follicles, glassy membrane remnants and atretic follicles. No *Corpora lutea*, *haemorrhagica* or *albicans* were observed (Table 1). Macroscopically, POCs were present in five of six (83%) cheetahs. They occurred singly in four of the five affected cheetahs (cheetahs 1-3, 5) and two POCs were present in one of the five affected cheetahs (Cheetah 4). All POCs were associated with the cranial flexure of the uterine tube near the tubal extremity of the right ovary. A *Rete ovarii* cyst was observed histologically in one cheetah (Cheetah 1). The largest follicle diameter recorded (Cheetah 1) was 2.5 mm.

3.3 Uterine histological changes

Mild histological changes were detected in the uteri of all six cheetahs (Figure 3) and consisted only of mild dilation of endometrial glands (Figure 2b,c). The occurrence and distribution of histological lesions differed significantly between animals (p < .001). In 3/6 cheetahs (Cheetahs 1, 5, and 6) a diffuse distribution pattern was observed, present in all seven uterine regions. In the remaining three cheetahs a multifocal distribution was detected in four of seven regions (Cheetahs 2 and 3) and two of seven regions (Cheetah 4), respectively (Figure 3). Endometrial glands were predominantly straight and sparse (Figure 2a).

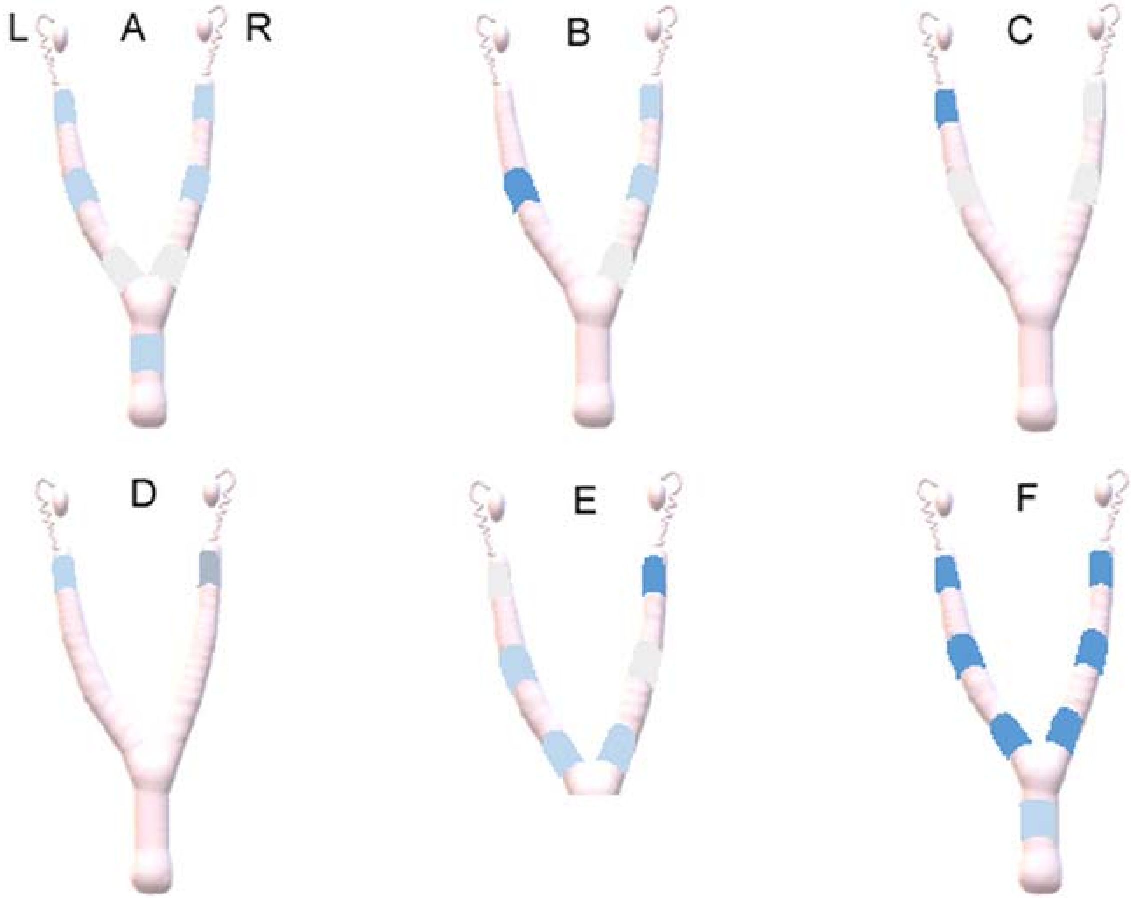
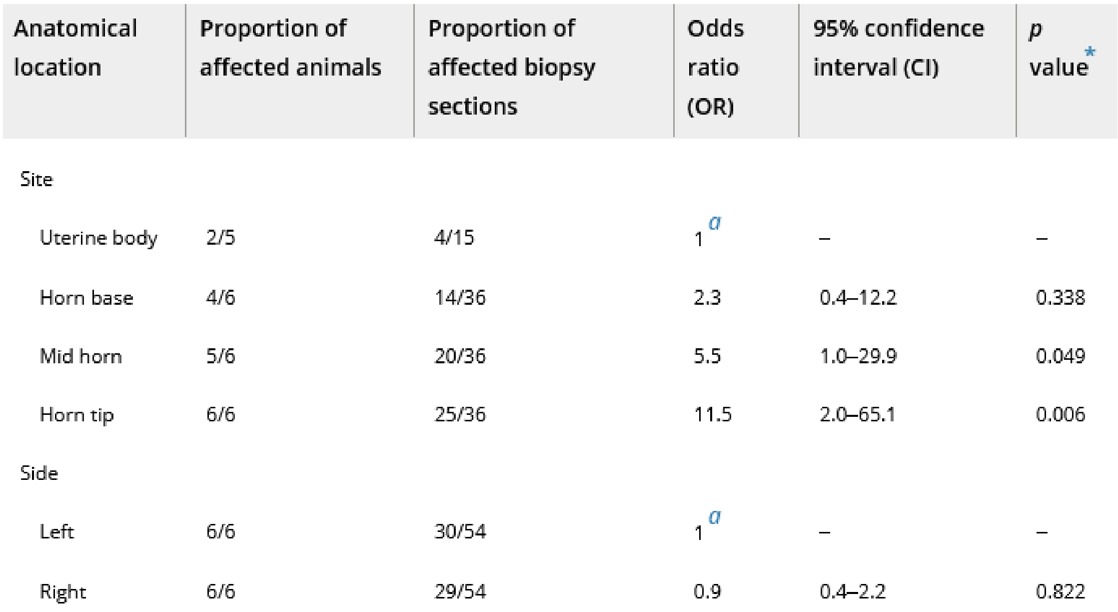


Figure 3. A heat map of endometrial gland dilation detected in six cheetah uteri collected post-mortem for the seven uterine regions sampled. (a) Cheetah 1; (b) Cheetah 2; (c) Cheetah 3; (d) Cheetah 4; (e) Cheetah 5 (note that the uterine body for Cheetah 5 was not collected); and (f) Cheetah 6

The likelihood of detecting endometrial gland dilation was lowest in the uterine body (2/5 animals, 4/15 sections), increased progressively in a cranial direction and was greatest at the uterine horn tip (6/6 animals, 25/36 sections) (Table 2). Compared to the uterine body, odds of dilation were significantly higher in the mid horn (OR = 5.5; 95% CI, 1.0-29.9; *p* = .049) and the horn tip (OR = 11.5; 95% CI, 2.0-65.1; *p* = .006). Odds of dilation were also significantly higher in the horn tip compared to the horn base (OR = 5.1; 95% CI, 1.6–15.6; *p* = .005). There was no significant difference in distribution between left and right horns, although the right horn tip was the only site where gland dilation was observed in all cheetahs (Figure 3).

Table 2. Mixed-effects logistic model of the distribution of endometrial glandular dilation distribution obtained by biopsy of uteri obtained postmortem from six nulliparous, Namibian cheetahs



Anatomical location	Proportion of affected animals	Proportion of affected biopsy sections	Odds ratio (OR)	95% confidence interval (CI)	p value[*]
Site					
Uterine body	2/5	4/15	1[a]	–	–
Horn base	4/6	14/36	2.3	0.4–12.2	0.338
Mid horn	5/6	20/36	5.5	1.0–29.9	0.049
Horn tip	6/6	25/36	11.5	2.0–65.1	0.006
Side					
Left	6/6	30/54	1[a]	–	–
Right	6/6	29/54	0.9	0.4–2.2	0.822

[a] Reference level.

[*] Significance of random effect for animal: $p < .001$.

3.4 Endometrial thickness

Endometrial thickness was significantly greater in the uterine horns (median, 648 µm; IQR, 577–707; range, 400–978) than in the uterine body (median, 575 µm; IQR, 512–629; range, 448–751; $p = .002$), but did not differ significantly between left (median, 645 µm; IQR, 554-685; range, 446–921) and right horns (median, 654 µm; IQR, 591–724; range, 400–978; $p = .127$). There was significant variation in endometrial thickness between cheetahs ($p < .001$), with the median within-animal measurements ranging from 547 to 772 µm.

4 DISCUSSION

Current practice is to obtain four uterine samples for histopathological study and to assume that results are representative of the entire uterus (St Louis Zoo, 2020). To the authors' knowledge this study is novel in that multiple regions of a felid uterus were systematically examined to critically validate this currently prevalent practice. Using apparently healthy captive cheetahs, we show that endometrial gland dilation is not evenly distributed throughout the uterus, but is significantly more prevalent in the cranial uterine horns. With regard to other lesions observed, gastritis is a common finding in captive cheetahs (Colburn et al., 2018) and is regarded as one of their major diseases in captivity (Terio et al., 2018). It has been reported as highly prevalent in the North American and Southern African captive cheetah populations with 99% of individuals affected. This prevalence is attributed to an elevated stress response (Colburn et al., 2018). The presence of gastritis in only 2/6 cheetahs may reflect decreased exposure to suspected environmental stressors in our study population, compared to other groups (Munson et al., 2005; Terio et al., 2018). Cheetahs fed muscle meat once a week have a reduced odds of developing chronic gastritis. The meat on the bone diet fed to the study population also decreases the risk of chronic gastritis developing (Whitehouse-Ted, Lefebvre, & Janssens, 2015). Dental attrition in five of the cheetahs is expected given the older age of the cheetahs (Steenkamp, Boy, van Staden, & Bester, 2018). The haemosiderosis of the meninges described in one cheetah was likely due to cranial trauma sustained as a juvenile from a motor vehicle accident. None of the mentioned pathology is expected to have any effect on the histology of the uterus or reproductive function.

All the uteri examined were in a nonpregnant state. Neither uterine signs of estrogenisation nor luteal structures in any of the ovaries were apparent. In most animals ovarian volumes ranged from 308.8 to 382.8 mm^3, previously shown to strongly correlate with anestrus in cheetahs (Schulman et al., 2015). Although one cheetah's ovarian volume (1,091 mm^3) was consistent with proestrus or estrus (Schulman et al., 2015) there were no associated uterine signs of estrogenisation. Most ovaries had immature follicles, with the largest follicle (2.5 mm) consistent with being a developing follicle (Wildt et al., 1993). A recent study that included 3-9-year old, captive female cheetahs from North American and Namibian establishments, confirmed short bursts of estrogenic activity interrupted in most cheetahs by intervals of apparent ovarian quiescence, particularly in winter and spring (Crosier, Comizzoli, Koester, & Wildt, 2016). In the above study acyclic periods were observed during approximately 1/3 of the 9-month observation period. Low-level estrogen waves were, however, detected during acyclic periods, indicating follicular cohorts undergoing development and atresia (Crosier et al., 2016). Estrogen peaks during cyclic periods averaged approximately 10 days (6.4–12.8 days) with shorter intervals in older cheetahs (Crosier et al., 2016). Cycle synchronicity is not reported in cheetahs (Terio, Marker, Overstrom, & Brown, 2003; Wielebnowski & Brown, 1998), and ovarian suppression does not occur in well-bonded groups of females (Terio et al., 2003). The current study population of six 7–10.5-year-old females had been grouped together for most of their lives, as management strategies at their sanctuary of origin aimed to group compatible individuals, suggesting that they were well bonded. Furthermore, their euthanasia coincided with mid-winter, previously associated with acyclicity (Crosier et al., 2016). All available information indicated that all these cheetahs were in anestrus with the possible exception of Cheetah 1 that may have been entering interestrus and was therefore classified as either anestrus or interestrus. Consideration must be given to the formalin shrinkage correction factor of 1.22 which is derived from human prostate studies (Partin et al., 1989) as no studies could be sourced on ovarian shrinkage correction factors. This factor may grossly overestimate ovarian shrinkage, in contrast human brain stem formalin shrinkage correction factor is 1.01–1.09 (Quester & Schröder, 1997), or underestimate ovarian shrinkage, a larger correction factor is used for prostate tumors by McNeal et al. (1986). The formalin shrinkage correction factor may have skewed results for Cheetah 1.

Paraovarian cysts, derived from the mesonephric duct or paramesonephric duct remnants, occur as single or multiple fluid filled structures associated with the cranial flexure of the ampulla located near the tubal pole of the ovary (Nielsen, Misdorp, & Mcentee, 1976; Schulman et al., 2015). They are not associated with reproductive consequences and are considered incidental findings (Schulman et al., 2015; Wachter et al., 2011). The observed high prevalence (88%) in the current study is consistent with that reported for a similarly aged group of cheetahs (Schulman et al., 2015).

The significance of the single observed ovarian rete cyst (Cheetah 1) is unknown (Gelberg, Mcentee, & Heath, 1984). This small structure was not compressing the cortex nor impinging on medullary structures. Its presence was therefore considered an incidental finding. The left and right cheetah ovarian volumes were not different in contrast with the African lioness with a larger and heavier left ovary (Hartman et al., 2012).

Since all but one of the cheetahs in this study were categorized as old (>9 years) and one as prime (6–8 years) the lack of age-related uterine pathology was remarkable. The current study observed none of the pathological changes associated with decreased reproductive performance. This is very different to previous reports from North American captive populations (Crosier et al., 2011; Munson et al., 2002). Despite a previous clinical ultrasonographic study, in which different aged females originating from the same facility reported a 19% prevalence of CEH (Schulman et al., 2015), these uteri from captive Namibian cheetahs did not show the same prevalence of age-related changes reported from North America (Crosier et al., 2011; Munson et al., 2002). These differences may reflect the difference in relative age of the two populations. The Munson et al. (2002) study does not provide ages for the 29 cheetahs evaluated, however, the Crosier et al. (2011) study population consisted of nine female cheetahs aged 9–15 years whereas the current study consists of five female cheetah aged 10–10.5 years and 1 cheetah aged 7 years. Although the presence of clinically significant uterine pathologies is likely to play a role in the poor reproductive performance of affected individuals (Crosier et al., 2011), they alone do not explain the overall poor reproductive performance of captive cheetahs, highlighting the multifactorial and complex nature of successful captive cheetah breeding.

Endometrial gland dilation was significantly more frequent in the uterine horns compared with the uterine body. Blood supply to and from the ovary and uterus of domestic cats is via the ovarian and uterine arteries and veins respectively; these vessels anastomose with each other in the region of the tip of the uterine horns (Aronsohn & Faggella, 1993), and blood supply in the cheetah appears similar (Penfold, 2019). Additionally, cheetahs have a prominent uterine tube blood vessel which anastomoses with the myometrial blood vessels in the tip of the uterine horn (Penfold et al., 2019). Higher plasma concentrations of ovarian-derived hormones flow from the ovary, some travelling via the vessels into the uterine horns. Ultimately, this circulating blood is diluted with systemic blood with lower plasma concentrations of ovarian hormones, due to the larger volume of distribution, supplied via the uterine arteries. Plausibly, parts of the uterus closer to the ovary would be influenced by a small but significant elevation in these hormone concentrations or ratios. This may arguably support the increased likelihood of detecting endometrial gland dilation in the horns. An alternative explanation is that there may be variation in the density of estrogen and progesterone or other hormone receptors between the horns and the body.

Endometrial thickness, although variable between animals, was essentially uniform throughout the uterine horns and within the uterine body of individual cheetahs. This uniformity probably reflects the absence of endometrial pathology and all cheetahs being of similar cyclical status, as demonstrated in the domestic cat during anestrus (Chatdarong, Rungsipipat, Axnér, & Forsberg, 2005). The significant variation in endometrial thickness between the uterine horns and uterine body of the cheetahs has to the authors' knowledge, not been reported before. A similar pattern was reported for smooth muscle development in the female reproductive tract of the cheetah where smooth muscle was much better developed in the region of the uterine horn than the uterine body (Penfold, 2019).

A limitation to this study is the small sample size, which may have limited our ability to detect associations. In addition, the paucity of pathological changes prevented any further insights into the distribution and classification of those conditions.

Half our study population showed a multifocal distribution and the other half a diffuse distribution of endometrial gland dilation. This may indicate that it initiates as a multifocal condition that progresses to a diffuse distribution in the uterine horns. The tips of the horns, particularly the right horn, since it was the only region to show at least one positive result for all the cheetahs, can be used to screen for its presence.

Our findings support examination of three sections obtained from the tip of the uterine horn as a screening method for detection of diffuse uterine changes in the cheetah. Due caution must be exercised when sampling this site to avoid accidental inclusion of the uterotubal junction with its papillary glands (Penfold et al., 2019) with a resultant erroneous diagnosis of CEH.

5 CONCLUSION

The tips of the uterine horns are the most likely region to show uterine gland dilation and can be used to screen for uterine changes in the cheetah. Since our results demonstrated that all cheetahs showed at least 1/3 samples positive for endometrial gland dilation when sampling the tip of the right uterine horn, it is advisable that three biopsy samples should be examined as early changes might be difficult to detect with a single sample.

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