Ultrasonic and laparoscopic evaluation of the reproductive tract in older captive female cheetahs (Acinonyx jubatus)

M.L. Schulman a,*, R.M. Kirberger b, A.S.W. Tordiffe c, L.L. Marker d, A. Schmidt-Küntzel d, M.J. Hartman b

a Section of Reproduction, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
b Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
c National Zoological Gardens of South Africa, Pretoria, South Africa
d Cheetah Conservation Fund, Otjiwarongo, Namibia

Article info
Article history:
Received 14 April 2015
Received in revised form 20 August 2015
Accepted 22 August 2015

Keywords:
Cheetah
Cystic endometrial hyperplasia
Deslorelin
Ovarian activity
Paraovarian cyst

Abstract
The study uniquely described the clinical value of transabdominal ultrasonography for monitoring features characterizing the estrous cycle in female cheetahs (Acinonyx jubatus). The reproductive tracts of 21 female, nulliparous, and relatively aged (median: 11 and interquartile range: 9.25–14 years) captive cheetahs resident on two sites in Namibia were assessed by transabdominal ultrasound. Subsequently, the ovarian findings on ultrasound were compared with direct visualization while performing laparoscopic sterilization. A combination of these observations supported by concurrent sampling for vaginal cytology and serum progesterone concentrations defined the estrous status of individual animals. At one site, six cheetahs had been implanted with the GnRH agonist, deslorelin as a contraceptive at least once within the preceding 11 years. On ultrasound, 31 uterine horns and 35 ovaries with discernible structures on 28 (86%) were visualized in the 21 cheetahs. The uterine body was difficult to visualize because of its intrapelvic location. Eleven of 19 uteri (58%) visualized showed endometrial edema suggestive of estrogenization. The uteri of four cheetahs (19%) showed evidence of mild cystic endometrial hyperplasia. Paraovarian cysts were seen on ultrasound (n = 21) and laparoscopy (n = 26) in 16 (76.2%) and 18 (85.7%) cheetahs, respectively. Ovarian volumes obtained from ultrasonographically determined dimensions predicted cyclic activity. Laparoscopy showed that 19 ovaries had discernible follicular structures. In the study population, 10 (47.6%) cheetahs were in proestrus or estrus; none in the luteal phase; and 11 (52.4%) in anestrus. Transabdominal ultrasound, in combination with serum progesterone concentrations and vaginal cytology, was used with acceptable accuracy to assess cyclic ovarian activity in captive cheetahs. A considerable proportion of this aged population showed ovarian activity and the prevalence of paraovarian cysts was notable. A history of prior deslorelin treatment was not associated with either reproductive activity or uterine pathology.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction
The increased availability and technological advances in ultrasonography together with improvements in hormone analyses and reproductive control methodologies have facilitated current reproductive management of free-range or captive wild carnivore populations, including cheetahs (Acinonyx jubatus). Behavioral observations, clinical and laboratory methods are all reported for estrous monitoring and defining the reproductive status of individual female cheetahs and lions [1–4]. These reports include hormonal...
analyses and the application of vaginal cytology in both free-ranging and captive populations and ultrasonography via transrectal and transabdominal approaches [5,6]. Descriptions of the normal reproductive tract, particularly with regard to imaging of the ovaries, are limited [1–3,7–9]. The utilization of ovarian imaging to observe changes in ovarian function in domestic felids is partially limited by the small size of the ovarian structures but has been reported as an adjunct to estrous monitoring in nondomestic felids [5,6,8,10–13]. Ultrasonography provides a noninvasive, practical, and relatively uncomplicated means to rapidly assess the reproductive status in both free-ranging and captive animals. Laparoscopy facilitates direct visualization of the ovaries and uterus, thus enabling evaluation of the dynamic changes associated with follicular and luteal phases of the estrous cycle as well as for the diagnosis (and staging) of pregnancy and pathologies of the reproductive tract of wild felids [5,6,13]. Additionally, uterine changes may also be indicative of the different stages of the reproductive cycle. Cheetahs in estrus display a thicker uterine wall with edematous, more anechoic bands compared to anestrous cheetahs which have thinner more echogenic uterine walls [6]. Transabdominal diagnostic ultrasound is particularly useful for in-the-field application in defining the reproductive status of both free-ranging and captive wild felid populations potentially providing a rapid alternative to laboratory-based methods for their reproductive management [5,6,14].

The reproductive cycles of many wild felids, including cheetahs, have been comprehensively reviewed [3]. The female cheetah, reproductively active throughout the year, shows relatively short cycles (7–21 days) characterized by estrogen surges that distinguish estrus (2–6 days) from interesting periods. There are four phases of the feld estrous cycle: proestrus, estrus, diestrus, and anestrus, which are distinguished by observation of ovarian structures. Proestrus and estrus are associated with the sequential development of visible follicles in the ovaries preceding the diestrus (or luteal phase) which is seen by the presence of one or more visible CL, often with a visible transition in development from a corpus hemorrhagicum (CH) [3]. Anestrus, the period of follicular quiescence, is associated with visibly nonfunctional follicular and luteal sites. [1,3]. Variable periods of ovarian quiescence, particularly in captive groups, are possibly due to socially induced and stress-associated ovarian suppression [3,15].

The GnRH analogue deslorelin is administered via a subcutaneous implant to downregulate reproductive activity as a safe and reliable contraceptive method in both male and female captive and free-ranging wild carnivores, including cheetahs [2,16]. Adverse effects have not been reported after long-term administration in treated female lions and tigers; however, similar data obtained from female cheetahs are currently limited [2,17,18].

Hormonal methods describing the estrous cycle, dependent on either serum or fecal sample–derived steroids, demonstrate cyclical elevations of estrogens differentiating anestrus from estrus. Currently, both induced (after mating) and, more rarely, spontaneous ovulations preceding the luteal phase (with associated elevations in progestogens after an estrogen surge) are recognized [1,3,7]. The pregnant luteal phase in the cheetah may be associated with a greater elevation in serum progesterone concentrations (SPCs) [2,9].

Vaginal cytology detects the effects of estrogenization resulting in an increased proportion of cornified vaginal epithelial cells and a clear background on fixed and stained vaginal epithelium smears to describe proestrus and estrus and is well described in the dog and cat [14,19]. The use of vaginal cytology in nondomestic felids, although not commonly performed, is reported as a valuable adjunct in determining cyclical activity [1,2,5].

It has been variously reported that captive breeding programs are typified by poor reproductive performance ascribed anecdotally to a plethora of causes including captive stress, lack of genetic diversity, poor husbandry conditions, and seasonal climatic changes [3,4]. Asymmetric reproductive aging has been reported in cheetahs similar to other captive populations of noncarnivore species such as elephants and rhinoceros in which older captive females demonstrate irregular estrous cycles or may cease cyclic activity [6,20,21].

The primary objective of this study was to assess the clinical value of transabdominal ultrasound observations of the reproductive tracts of 21 nulliparous, aged, captive cheetahs via comparison with direct laparoscopic visualization at the time of sterilization. These methods in combination with data derived from concurrent measurement of SPC and vaginal cytology were used to define the reproductive status of the cheetahs.

2. Materials and methods

2.1. Animals

All cheetahs in this study were obtained as orphaned wild cubs and maintained thereafter as nonreleasable wild cheetahs by two independent cheetah conservation organizations (sites A and B) in northern Namibia. The females were kept in small groups of two to five animals under free-range conditions in large, fenced, bushveld camps (ranging from 3 to 50 ha) separated by at least a fence line or roadway from similar small groups of males. Their diet consisted mainly of donkey and horse meat on the bone with a commercial vitamin and mineral supplement (Predator Supplement; V-Tech, Centurion, South Africa).

Current Namibian legislation requires surgical sterilization of all captive female carnivores [16]. The University of Pretoria was requested to initiate a surgical sterilization program at the above two conservation sites to comply with this legislation. The project was approved by the University’s Animal Use and Care Committee and Research Committee (protocol number: V014-14) and conducted under the terms of permit 1919/2014 from the Namibian Ministry of Environment and Tourism.

Twenty-one sexually mature, nulliparous, female cheetahs were included in a laparoscopic surgical sterilization trial comparing ovarioectomy to salpingectomy during July 2014 [22]. The number, age (median and interquartile range [IQR]), body weight (median and IQR) of the animals at both sites and their combined data respectively were as follows:
Site A: n = 11, 11 (IQR: 9.25–12 years), 33.2 (IQR: 31.6–33.4 kg).
Site B: n = 10, 13 (IQR: 10–14 years), 32.75 (IQR: 30.0–36.0 kg).
Combined population: n = 21, 11 (IQR: 9.25–14 years), 33.2 (IQR 31.2–35.0 kg).

Records showed that at site A, six cheetahs had received at least one 4.7-mg implant of the GnRH agonist deslorelin (Suprelorin; Virbac, Milperra, New South Wales, Australia) within the preceding 11 years (Table 1).

All cheetahs were examined under general anesthesia during a 9-day observation period. The cheetahs were fasted 24 to 48 hours preoperatively but had free access to water. Cheetahs at site A were immobilized by remote injection using a dart gun with combinations of tiletamine and zolazepam (Zoletil; Virbac, Halfway House, South Africa) and medetomidine (Domitor; Pfizer Animal Health, Sandton, South Africa). At site B, the cheetahs were trained to enter a capture cage in which the above drugs were injected intramuscularly. The cheetahs were maintained on isoflurane (Isofor; Safeline Pharmaceuticals, Johannesburg, South Africa). Morphine sulfate (Fresenius Kabi, Port Elizabeth, South Africa) was given for pain control during surgery. After the procedure, anesthesia was antagonized with atipamezole (Antisedan; Pfizer Animal Health). For postoperative analgesia, 0.3 mg/kg of meloxicam (Metacam, 5-mg/kg; Boehringer Ingelheim, Randburg, South Africa) was administered subcutaneously before anesthetic recovery. One cheetah at site A had a concomitant intra-abdominal granuloma with associated abdominal effusion, secondary to an abdominally located thorn. This granuloma was removed laparoscopically [23].

2.2. Ultrasonography

Each cheetah underwent a dorsally recumbent trans-abdominal ultrasound examination of its reproductive tract. A portable ultrasound machine was used with a 3- to 6-MHz convex array transducer for the general abdominal examination and a 5- to 10-MHz linear array transducer for evaluation of the reproductive tract (Mindray Model M7 Vet; Shenzhen Mindray Bio-Medical from Lomaen, Johannesburg, South Africa). The uterine body, horns, ovaries, and surrounding structures were examined, and their associated dimensions, echogenicity, wall layering, luminal content, and the presence of cyclical activity were recorded. The uterine horns were categorized as being either cyclically active or inactive [6], and the average diameter of both horns and the presence of uterine edema (associated with estrogenization) were compared retrospectively to the designated reproductive status of individual cheetahs to determine a difference in size between the cyclically active and inactive groups. Round intraovarian visualized structures were classified as follicles if they were anechoic and had acoustic enhancement and as periovulatory structures which included late, luteinizing follicles or CL (slightly echogenic with minimal acoustic enhancement) and CL (slightly hyperechoic to the surrounding ovarian tissue with no acoustic enhancement) [10]. The location of the ovaries relative to the ipsilateral kidney and surrounding structures was recorded. The ovarian size and

Table 1

<table>
<thead>
<tr>
<th>Site and animal ID &amp; age (y)</th>
<th>SPC (nmol/L)</th>
<th>Vaginal cytology estrogenized (Y/N)</th>
<th>Reproductive status</th>
<th>Uterine horns US visible (Y/N)</th>
<th>Uterine horns US edema (Y/N)</th>
<th>Left ovary</th>
<th>Right ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (6)a</td>
<td>0.222</td>
<td>N</td>
<td>anes</td>
<td>Y/N</td>
<td>N/Y</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A2 (12)</td>
<td>0.590</td>
<td>N</td>
<td>anes</td>
<td>Y/Y/Y</td>
<td>3/0/0</td>
<td>2/0/1</td>
<td>2/2/1</td>
</tr>
<tr>
<td>A3 (12)</td>
<td>0.852</td>
<td>N</td>
<td>anes</td>
<td>Y/N/N</td>
<td>0/0/0</td>
<td>1/1/0</td>
<td>0/0/1</td>
</tr>
<tr>
<td>A4 (9)</td>
<td>2.185</td>
<td>N</td>
<td>anes</td>
<td>Y/N/N</td>
<td>2/0/1</td>
<td>1/0/0</td>
<td>0/0/1</td>
</tr>
<tr>
<td>A5 (9)</td>
<td>0.413</td>
<td>N</td>
<td>anes</td>
<td>N/N</td>
<td>—/0/0</td>
<td>1/0/0</td>
<td>0/0/1</td>
</tr>
<tr>
<td>A6 (11)a</td>
<td>1.463</td>
<td>N</td>
<td>anes</td>
<td>Y/Y/Y</td>
<td>N/Y/N</td>
<td>0/0/0</td>
<td>0/0/1</td>
</tr>
<tr>
<td>A7 (11)a</td>
<td>1.176</td>
<td>N</td>
<td>anes</td>
<td>Y/N/Y</td>
<td>ms/mul</td>
<td>4/4</td>
<td>2/2</td>
</tr>
<tr>
<td>A8 (11)a</td>
<td>1.263</td>
<td>Y</td>
<td>pro/est</td>
<td>Y/Y/Y</td>
<td>1/1/2</td>
<td>3/2/0</td>
<td>2/0/0</td>
</tr>
<tr>
<td>A9 (7)</td>
<td>0.801</td>
<td>N</td>
<td>anes</td>
<td>Y/N/N</td>
<td>0/0/2</td>
<td>0/0/1</td>
<td>1/1/1</td>
</tr>
<tr>
<td>A10 (12)</td>
<td>2.445</td>
<td>N</td>
<td>anes</td>
<td>Y/N/N</td>
<td>ms/mul</td>
<td>3/3</td>
<td>1/1/1</td>
</tr>
<tr>
<td>A11 (14)</td>
<td>2.462</td>
<td>Y</td>
<td>pro/est</td>
<td>N/N</td>
<td>—/1/0</td>
<td>2/1/2</td>
<td>2/2/2</td>
</tr>
<tr>
<td>A12 (14)</td>
<td>7.058</td>
<td>Y</td>
<td>pro/est</td>
<td>Y/Y/Y</td>
<td>ms/mul</td>
<td>1/1/0</td>
<td>0/0/1</td>
</tr>
<tr>
<td>A13 (14)</td>
<td>4.093</td>
<td>Y</td>
<td>pro/est</td>
<td>Y/Y/Y</td>
<td>6/0/10</td>
<td>mul/2</td>
<td>0/0/0</td>
</tr>
<tr>
<td>A14 (14)</td>
<td>2.760</td>
<td>undefined</td>
<td>anes</td>
<td>Y/Y/Y</td>
<td>2/mul</td>
<td>1/2/3</td>
<td>0/1/1</td>
</tr>
<tr>
<td>B15 (10)</td>
<td>2.784</td>
<td>Y</td>
<td>pro/est</td>
<td>Y/Y/N</td>
<td>4/1/1</td>
<td>1/1/1</td>
<td>0/1/0</td>
</tr>
<tr>
<td>B16 (11)</td>
<td>1.031</td>
<td>Y</td>
<td>pro/est</td>
<td>Y/Y/Y</td>
<td>1/1/0</td>
<td>5/0/2</td>
<td>2/2/2</td>
</tr>
<tr>
<td>B17 (8)</td>
<td>1.526</td>
<td>Y</td>
<td>pro/est</td>
<td>Y/Y/Y</td>
<td>3/2/0</td>
<td>1/1/1</td>
<td>0/0/1</td>
</tr>
<tr>
<td>B18 (8)</td>
<td>1.359</td>
<td>N</td>
<td>anes</td>
<td>N/Y/N</td>
<td>—/0/0</td>
<td>0/0/1</td>
<td>0/0/1</td>
</tr>
<tr>
<td>B19 (14)</td>
<td>0.352</td>
<td>Y</td>
<td>pro/est</td>
<td>Y/Y/Y</td>
<td>ms/mul</td>
<td>1/1/1</td>
<td>2/2/3 mul</td>
</tr>
<tr>
<td>B20 (12)</td>
<td>6.411</td>
<td>pro/est</td>
<td>Y/Y/Y</td>
<td>4/4/3</td>
<td>0/0/1</td>
<td>1/1/1</td>
<td>1/1/1</td>
</tr>
<tr>
<td>B21 (14)</td>
<td>1.849</td>
<td>pro/est</td>
<td>Y/N/N</td>
<td>—</td>
<td>2/2/0</td>
<td>0/0/1</td>
<td>0/0/1</td>
</tr>
</tbody>
</table>

Abbreviations: A, site A; anes, anestrus; B, site B; pro/est, proestrus or estrus; Lap, laparoscopy; ms, multiple small follicles (<2 mm diameter); mul, multiple cysts or follicles; N, no; PO, paraovarian (ovary not seen); SPC, serum progesterone concentration; US, ultrasound; Y, yes.

a Prior deslorelin treatment.
structure were compared to direct visualization during the ensuing laparoscopic procedure. Single or multiple, round, anechoic cystic structures in close proximity to the ovaries were defined as parovarian (PO) cysts.

2.3. Laparoscopy

Immediately after the ultrasonographic examination, each cheetah underwent a single-incision laparoscopic surgery starting in dorsal recumbency. A 5-mm 0° endoscope (Hopkins Optik, Karl Storz GmbH & Co. KG, Tuttingen, Germany) was inserted for visual inspection of the abdominal cavity. The cheetahs were tilted 45° to the left followed by 45° to the right, and the right and left ovaries and associated structures, respectively, were identified. Digital images were captured using a H3 camera head and HD camera Image 1 HUB with a Xenon 300 nova light source, AIDA data recording device, and a 26-inch screen (Karl Storz GmbH & Co. KG).

Thereafter, all cheetahs underwent bilateral salpingectomy (n = 10) or ovariectomy (n = 11). The authors retrospectively examined the laparoscopic images obtained and described both of the observed ovarian structures, follicles, peri-ovulatory or luteal structure (either CH or CL), and PO cysts and compared them with the ultrasonographic images obtained.

2.4. Reproductive status

2.4.1. Vaginal cytology

Vaginal smears were obtained from each cheetah immediately before ultrasonography after passage of a sterilized, clear, perspex tube speculum of 12 mm diameter via the vulva and vestibulum. Thereafter, a sterile cotton-tipped swab attached to a stainless steel wire was passed through the tube until making contact with the cranial vaginal mucous membrane, rotated several times, retrieved, and rolled gently on a glass microscope slide that was air dried and stained with Diff-Quik (Kryoquik; Kyron Laboratories, Benrose, South Africa) before permanent mounting using Entellan (Merck, Halfway House, South Africa) and a glass coverslip. The slide was examined microscopically to describe the presence and percentage of superficial epithelial cells expressed as the superficial cell index and the clarity of the background as an indicator of the presence or absence of estrogenization [1,2].

2.4.2. Hormone assays

Serum samples were collected for SPC determination as an indication of peri-ovulatory or luteal status by RIA [1,2]. The samples were stored at −40 °C until transfer to the Immunoncontraception Laboratory, Faculty of Veterinary Science, University of Pretoria followed by thawing for assay using progesterone RIA kits (Progesterone Coat-a-Count; Diagnostic Products) as previously described [18].

2.4.3. Ovarian structures

The ovaries were observed in situ by a combination of ultrasonography and laparoscopy. The ultrasonographic ovarian dimensions (length, width, and height) were measured for each observed ovary, and the volumes were calculated using the method of Pavlik et al. [24] as an indicator of cyclic ovarian activity. The calculated ovarian volumes from the individual cheetahs in which both ovaries were visualized (n = 16) were compared with their reproductive status as assessed from the SPC and vaginal cytology data. The ultrasonographic characteristics and dimensions of all observed ovarian structures (follicles, CH, and CL) and adnexal PO cysts were recorded. Ovarian activity on laparoscopic visualization was defined by the observation of prominent structures. Clear, vesicular-appearing structures were recorded as follicles. The peri-ovulatory or luteal phases were defined by observation of one or more prominent, somewhat irregular yellowish structures with evidence of increased vascularization [13,25].

2.4.4. Categorization of cyclic activity

On the basis of the combination of vaginal cytology, SPC measurements, and the observed ultrasonographic and laparoscopic ovarian structures, the cheetahs were categorized as being cyclically active (proestrus or estrus and diestrus) or inactive (anestrus) [10].

2.4.5. Deslorelin treatment

At site A, six cheetahs received one or more number (range: 1–3) of treatments between 2003 and 2013 with 4.7-mg deslorelin implants (Suprelorin; Virbac) with the most recent administration in cheetah no. 1 approximately 12 months before examination. The effects of this treatment on uterine pathology and reproductive activity were compared with the nontreated study populations from both sites (n = 15).

2.5. Statistical analysis

Data were tested for normality using the Kolmogorov–Smirnov test. Nonparametric data were reported as median and IQR according to Tukey’s hinges. The Wilcoxon single-rank test was used to compare contralateral ovarian volume. The Mann–Whitney U test was used to compare total uterine volume and uterine horn diameter in cyclically active and inactive cheetahs. The mean number of follicles of diameter 2 mm or greater detected in each cheetah by ultrasound or laparoscopic examination was compared between these modalities using a paired samples t test. Proportions of cyclically active and inactive animals that showed uterine edema were analyzed using a two-sided Fisher’s exact test. Similarly, a two-sided Fisher’s exact test was used to determine the ratios of deslorelin-treated animals that showed uterine pathology and additionally their reproductive activity. Sensitivity and specificity data were obtained using empirical receiver operating characteristic curves, and a cutoff value for 100% specificity was determined. A linear regression model with Bonferroni correction was used to determine the function between ovarian volume and uterine horn diameter. All analyses were performed with the SPSS v.17 (IBM, New York, USA) statistical software package. Statistical significance was defined as P < 0.05 in all cases, with the exception of the regression model (P < 0.025).
3. Results

3.1. Ultrasonography

The uterine body dorsal to the bladder was difficult to detect as the caudally converging uterine horns entered the pelvic canal at the pubic rim before joining and could not be seen further caudally because of pubic bone acoustic shadowing. The cervix and vagina were thus also not observed. At least one ovary or uterine horn was seen in all but two cheetahs (Table 1). Thirty-one of a possible 42 uterine horns (76.2%) were seen either by imaging transversely cranial to the pubis and detecting the horns dorsal or adjacent to the bladder, or by following the ovaries caudally. Eleven cheetahs had edematous uterine walls, usually with visible wall layering and thus likely to be in either proestrus or estrus (Fig. 1A), whereas eight had nonedematous uterine walls, likely to be associated with anestrus (Fig. 2A; Table 1). In cheetahs categorized as cyclically active (i.e., proestrus or estrus), uterine horns were seen in 17 of 20 (85%), and 14 of 22 horns (64%) were seen in those categorized as inactive (i.e., anestrus; Table 1). There was no statistical difference in uterine horn diameter between cyclically active (4.7, IQR: 4.4–5.2 mm; n = 9) and inactive individuals (4.2, IQR: 3.7–5.2 mm; n = 9; \( P = 0.340; U = 29 \)) or between cyclic reproductive activity and uterine edema (\( P = 0.250; \) Table 2). Ultrasonography identified five cheetahs (23.8%) with features consistent with mild uterine pathology. Mild cystic endometrial hyperplasia (CEH) with one to three ultrasonographically visible intramural cysts (largest = 5.9 mm diameter) was present in four cheetahs (19.0%) aged 11, 12, 14 (2x) years (Fig. 3A). A small accumulation of intraluminal uterine fluid, believed to be a mucometra, was seen in one 12-year-old cheetah. Hydrosalpinx, which mimicked small ovarian or PO cystic structures, was present in four cheetahs (19%) aged 8, 16, 19, 20 years (Fig. 1C, D), two of which additionally showed CEH.

Thirty-five of a possible 42 ovaries (83.3%) were seen in 19 cheetahs with none in two and unilaterally in three cheetahs (Table 1). On the left side, colonic gas and, on the right side, small intestines occasionally interfered with attempts to image the ovaries. The left ovary was generally easier to find than the right ovary where small intestines often hampered ovarian identification. The ovary was usually located immediately caudal to the region where the caudal hyperechoic edge of the caudal perirenal fat joined the ventral hyperechoic surface edge of the iliopsoas muscle fascia at an angle of approximately 60° as described in lions [5]. In some cheetahs, the ovaries were more readily found by tracing the uterine horns cranially.

Structures classified as follicles were seen on 28 ovaries (Fig. 1B), and those structures classified as periovulatory follicles were seen on six ovaries (Fig. 3B). The largest follicular structure observed was 6.2 mm. The mean number of follicles detected per cheetah which included visualization of both ovaries was 2.7 ± 2.6. Seven ovaries lacked discernible cyclic activity (Fig. 2B).

Fig. 1. Cheetah 8, a 11-year-old animal in proestrus or estrus. Ultrasonographic (A–C) and laparoscopic images (D): (A) edematous right uterine horn with wall layering. Walls were 2.6 and 3.0 mm thick (between cursors) giving a total wall diameter of 5.6 mm, (B) left ovary with 6.2-mm-diameter anechoic follicle (between cursors) demonstrating acoustic enhancement. Anechoic structure to the left of the follicle was a paraovarian cyst. (C) Paraovarian cyst cranial to the left ovary 8.7 mm in diameter (between cursors). Anechoic structure (*) is a section of left fallopian tube with hydrosalpinx, (D) left ovary with large paraovarian cyst cranial to the ovary and hydrosalpinx seen prominently to the right of the forceps and superimposed on the ovary (*). Cranial is to the left.
Single or clusters of multiple PO cysts (n = 21; Table 1) in 16 (76.2%) cheetahs were observed on ultrasound; these were detected cranial to the ovaries with the exception of two cheetahs with unilateral PO cysts caudal to the ovary (Figs. 1C, 2C, 4). Ten sites had one or more cysts, and one site was associated with more than 10 cysts. The PO cysts varied in diameter up to a maximum of 18.9 mm (Fig. 4). These relatively large, anechoic cystic structures facilitated ultrasonographic detection of the ipsilateral ovaries.

3.2. Laparoscopy

The laparoscopic ovarian data are given in Table 1, and the images are presented in Figures 1D, 2D, and 3C. Comparing ultrasound with laparoscopy showed that laparoscopy visualized all ovaries including the seven not seen on ultrasound in five cheetahs (Figs. 1C, 2C, 4). Ten sites had one or more cysts, and one site was associated with more than 10 cysts. The PO cysts varied in diameter up to a maximum of 18.9 mm (Fig. 4). These relatively large, anechoic cystic structures facilitated ultrasonographic detection of the ipsilateral ovaries.

3.3. Ovarian volumes

Sixteen cheetahs had bilaterally observed ovaries; there was no difference between left (513, IQR: 408–782 mm³) and right (521, IQR: 376–644 mm³) ovarian volumes (P = 0.642); however, total ovarian volumes for those categorized as cyclically active, i.e., proestrus or estrus (n = 9) were significantly greater (1322, IQR: 1024–1363 mm³) than those in anestrus (n = 7; 906, IQR: 387–964 mm³; P = 0.005; U = 6). Total ovarian volumes greater than 1200 mm³ had a specificity of 100%, a sensitivity of 67%, and a 1.0 positive predictive value for cyclic ovarian ultrasound had corresponding structures on laparoscopy, 11 ovaries with follicles visible on ultrasound lacked corresponding structures on laparoscopy, two ovaries with follicles visible on laparoscopy showed none on ultrasound, the mean number of follicles detected per cheetah including both ovaries was 2.3 ± 3.1, and PO cysts were identified in two additional cheetahs on laparoscopy.

Nine of the 10 cheetahs categorized with cyclic ovarian activity had visible signs of cyclic ovarian activity apparent on both ultrasonography and laparoscopy, with follicles or periovulatory structures seen on 10 ovaries, and these modalities agreed 100% on the identification of the type of activity (Table 1). There was no difference between the two modalities in detecting ovarian follicles (P = 0.611; t = 0.517).

### Table 2

Ovarian cutoff volume for cyclic reproductive activity.

<table>
<thead>
<tr>
<th>Ovarian volume</th>
<th>Cyclic</th>
<th>Noncyclic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1200 mm³</td>
<td>3</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>&gt;1200 mm³</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>7</td>
<td>16</td>
</tr>
</tbody>
</table>

Sensitivity: 67%  Speciﬁcity: 100%
activity (Table 2). The area under the receiver operating characteristic curve was 0.905 (95% confidence interval: 0.75–1.00) which indicates a high diagnostic accuracy. There was a weak relationship between total ovarian volume and uterine horn diameter ($r^2 = 0.306$; $P = 0.040$).

3.4. Reproductive status

The reproductive status assignation of the study population’s cheetahs showed 10 (47.6%) cheetahs consistent with proestrus or estrus (follicular or periovulatory phase) and 11 (52.4%) cheetahs with anestrus (Table 1). None demonstrated a postovulatory luteal phase as evidenced by SPCs.

3.5. Deslorelin treatment

One treated cheetah (No. 8) with a last treatment date approximately 11 years before the study showed obvious reproductive activity (Table 1). Neither the ratios of cheetahs that showed either reproductive activity ($P = 0.149$; Table 3) or uterine pathology, as detected by ultrasound ($P = 0.262$; Table 4), differed subsequent to treatment with this chemical contraceptive implant.

4. Discussion

The study populations’ median age placed them within a cohort ideal for evaluating the effects of aging reportedly associated with markedly decreased reproductive competency in which older cheetahs were classified as 9 to 15 years of age [13]. Ultrasonographic evaluation of the reproductive tract, uniquely enhanced by the concurrent direct laparoscopic visualization of structures, provided a valuable insight into the reproductive status of cheetahs. Laparoscopically, the absence of an enveloping ovarian
bursa obscuring the felid ovary (as found in Canidae) enabled complete visualization of the ovary, its associated structures, and adnexa. Laparoscopic visualization of the cheetah ovary had previously only been reported for assessing the effects of exogenous gonadotropin administration and oocyte retrieval [13].

Ovaries lacking structures associated with normal cyclic activity proved relatively difficult to visualize on ultrasonography; however, most had follicles and additionally, many had associated adnexal PO cysts facilitating their localization. After ovarian localization, the adjacent ipsilateral uterine horn was generally observed facilitating the process of following the uterine horns further caudally. The relatively low body weight of the cheetah together with numerous active ovaries facilitated identification and accurate ultrasonographic data collection when compared to larger wild felids. Comparing the cheetahs to a similar ultrasonographic study in sexually mature lions, 76.2% versus 58% (uterine horns) and 83.3% versus 46% (ovaries) in cheetahs and lions, respectively, were recorded [5]. The greater detection rate in cheetahs compared to lions is most likely due to cheetahs being smaller, weighing less, and having less intra-abdominal fat. These factors allow higher frequency transducers to be used with associated improved resolution. In this study, the presence of PO cystic structures primarily, and follicles secondarily, were the most important factors enhancing ovarian visibility and abdominal fat did not impede the visualization of reproductive structures. The transabdominal route utilizes widely available equipment and facilitated optimal imaging of the ovaries and adnexal structures in cheetahs in the field, a primary aim of this present study. Despite a paucity of reports in cheetahs, transrectal ultrasonography may provide enhanced visualization of the uterus in particular [6], although its requirement for a specialized transducer and potential risks of this invasive approach may outweigh its potential benefits when applied in the field. To optimize visibility of the described structures, it is advisable to use appropriate equipment and experienced ultrasonographers.

Although cyclically active and inactive animals were readily distinguished, the precise differentiation of follicular (including proestrus, estrus, periovulatory) and post-ovulatory (luteal) status was less straightforward. Certainty of detection of ovulation via definition of a postovulatory CL and hence precise determination of estrous status by ultrasonography alone may be controversial as was supported by the present study [10,12]. The predictive value of ovarian volumes for determining cyclic activity in cheetahs was supported. The absence of any luteal phase in the present study supported the reported rarity of spontaneous ovulation in captive cheetahs when compared with lions, despite the relative close proximity of male cheetahs [13,5,7]. Ultrasonography of uterine horn diameter and uterine wall edema were not significant predictors of cyclic activity. The uterine horns in cheetahs showing cyclic activity (85%) were however more readily visualized than horns in inactive cheetahs (64%).

Previous studies reporting effects of GnRH agonists including deslorelin in various domestic and nondomestic felids were limited by indirect methods such as laboratory-based serologic and fecal steroid metabolite assays for evaluating cyclic ovarian activity when compared with direct ultrasonographic evaluation of ovarian and uterine appearance [2,13,14]. Inconsistent responses to GnRH agonists may make ultrasonography an ideal modality for monitoring any indication for additional administration of these agents in captive wild felids under field conditions [14]. The present study supported the previously reported absence of any obvious adverse effects despite repeated deslorelin administration [2].

This study supported a previous report describing ovarian activity in older nulliparous female cheetahs in which age had no apparent influence on ovarian volumes [13]. Relatively few cheetahs showed ultrasonographic evidence of either CEH or mucometra, correlating with a previous report in a similar-age cohort in which these findings did not differ among three age categories [13]. Previous captive cheetah uterine histologic studies have found an increasing age associated with increasing incidences of uterine degenerative changes with variable effects of parity [13,26].

The relatively high prevalence of adnexal PO cystic structures (76.2%) in this study population in either a cranial location on the suspensory ligament (87.5%) or a caudal location between distal uterine horn and ovary (12.5%) associated with the reproductive tract was notable. Paraovarian (also paratubal) cysts are benign intraperitoneal cysts arising from the broad ligament and are mesonephric duct remnants [27–29]. Paratubal cysts additionally include paramesonephric remnants, the small hydatid cysts of Morgagni attached to the fimbriae of the fallopian tube [27,28]. Their significance in cheetahs is undefined, and reportedly, they do not appear to influence fertility [6].

The present study’s ultrasonographic identification of numerous incidents of adnexal cystic structures including hydrosalpinx must be considered in their differentiation from normal ovarian structures in similar populations. Paraovarian cysts have been described in domestic animal species including sheep and wild felids including cheetahs, lions, and tigers, with parauterine cysts being reported in the lion [5,6,8,30]. Ultrasonography is the primary and preferred imaging modality for anechoic cystic structures visualized in women, and these include follicles, functional follicular cysts, early CLs, PO cysts, and the convoluted

### Table 3

<table>
<thead>
<tr>
<th>Uterine horn edema</th>
<th>Yes</th>
<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclic (count)</td>
<td>7</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Non-cyclic (count)</td>
<td>4</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Total (count)</td>
<td>11</td>
<td>8</td>
<td>19</td>
</tr>
</tbody>
</table>

### Table 4

<table>
<thead>
<tr>
<th>Uterine pathology</th>
<th>Pathology</th>
<th>No pathology</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contracepted or not</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contracepted (count)</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Not contracpted (count)</td>
<td>10</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>Total (count)</td>
<td>16</td>
<td>21</td>
<td>37</td>
</tr>
</tbody>
</table>
appearing hydrosalpinx [31]. In our study, there was no difference in the number of follicles observed between ultrasound and laparoscopic examination. Adnexal cysts are usually separated from the ovary but if closely adjacent may be mistaken for follicles. In women, they occur in middle age, tend to be benign but may predispose to ovarian torsion [31]. Concomitant laparoscopy was ideal for verification of these and other ultrasonographic findings. A limitation of this study was that of laparoscopy in clearly observing smaller intraovarian follicles of less than 2 mm in diameter. This may contribute to discrepancies but concurrent application of other clinical and laboratory modalities will not appreciably bias the ability to categorize cyclic activity.

Sterilization using various laparoscopic techniques is described in tigers and lions and similarly proved an appropriate surgical technique for captive cheetahs, being both rapid and with low complication rates that facilitated appropriate surgical technique for captive cheetahs, being described in tigers and lions and similarly proved an appreciably bias the ability to categorize cyclic activity.

References
[27] Palmieri C, Schiavi E, Saldaa LD. Congenital and acquired pathology of the tubular tract in this aged population. These procedures offer potential benefits in assessment of individual reproductive function, including subfertility, and assisted reproduction techniques in similar populations of cheetahs.

Acknowledgments
The authors extend their thanks to E Monnet, J Standen, F Stegman, and staff of the AfriCat Foundation and CCF who were involved with the ultrasonographic and laparoscopic procedures as well as handling the animals. Thanks to Karl Storz for sponsoring laparoscopic equipment and Lomaen Medical for sponsoring the Mindray ultrasound machine. Special thanks go to the owners and directors of the AfriCat Foundation and CCF in Namibia for supporting this research project and allowing it to take place on their respective properties. M.L. Schulman and R.M. Kirberger contributed equally to the article.

References