Responsiveness of the cheetah (*Acinonyx jubatus*) ovary to exogenous gonadotropins after preemptive oral progestin treatment

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Abstract

Control of ovarian function in cheetahs is sub-optimal, which currently limits the integration of assisted reproductive techniques into the genetic management of that endangered species. The objective of this study was to determine the effect of preemptive progestin treatment on the quality of ovarian responses after exogenous gonadotropin stimulation in cheetahs. Adult females received either 1) 200 IU equine chorionic gonadotropin (eCG) followed with 3,000 IU porcine luteinizing hormone (pLH) (intramuscular route) (n = 5; control group) or 2) similar eCG/pLH administration preceded by a 7-day treatment with oral progestin (0.1 mg/kg altrenogest; ALT group; n = 7). At 42 h post-pLH administration, a series of metrics was assessed via laparoscopy (number of follicles ≥ 2 mm, number of corpora lutea, oviduct and uterine cornua diameter and overall vascularization). Concentrations of fecal estradiol, progesterone and glucocorticoid metabolites (FEM, FPM, and FGM, respectively) were measured by enzyme immunoassay for 3 wk before ALT treatment (Period 1), 7 d during ovarian suppression period (Period 2), throughout eCG/LH treatment and laparoscopy (Period 3), and 6 wk following laparoscopy (Period 4). Overall, nine out of 12 cheetahs (4/5 in control and 5/7 ALT group) had freshly-formed corpora lutea at the time of laparoscopy. Mean follicle and corpora lutea numbers in the control versus ALT group were not different (P > 0.05). Overall measurements and vascularization scores also did not differ (P > 0.05) among groups. FEM average concentrations increased (P ≤ 0.05) in response to eCG for the ALT-treated females between Periods 2 and 3 and were sustained during Period 4. However, FEM average concentrations did not vary (P > 0.05) for control females throughout Periods 1-4. Post-ovulatory FPM average concentrations (Period 4) did not differ (P > 0.05) between the ALT-treated females and controls. FPM average concentration from both groups increased in Period 4 compared to Periods 1-3 (P ≤ 0.05). Females receiving the ALT treatment also had lower (P ≤ 0.05) FGM metabolite average concentrations than control females during ovarian suppression (suggesting adrenal suppression). Collective results suggest that ovarian response to gonadotropin treatment in the cheetah was improved following oral progestin administration due to the normative increase in estradiol following stimulation for these females compared with control. This treatment should lead to more effective timed assisted reproduction procedures for this species.

Keywords: endangered species; progestin; fecal steroid metabolites; gonadotropin stimulation; ovarian suppression

Introduction

Among the genus *Acinonyx*, cheetahs (*Acinonyx jubatus*) are the only living species, and are currently classified as vulnerable by the International Union for Conservation of Nature [1]. Numbers are declining throughout historic ranges in western, central and northern Africa, primarily due to habitat loss and population fragmentation [2]. Captive management programs using both natural breeding and, to a lesser extent, assisted reproductive techniques (ARTs), such as sperm cryopreservation and artificial insemination (AI), have proven to be key elements of cheetah survival, generating genetically valuable offspring that can serve as insurance populations [3, 4, 5]. To date, AI techniques combined with sperm cryopreservation have been widely applied to felid population management [6] although pregnancy success often is limited and varies across the felid taxa (e.g., 33% to 78% success in domestic cats [7,8,9]; 22% in cheetahs [10]; 20% in tigers [11]; 0% in fishing cats [12] and recently 25% in clouded leopard [13]).

One biological challenge of cheetahs is that females have inconsistent estrous cycles, which vary in length from 5 to 28 days, and are interrupted by acyclic periods that often last for more than 30 days [14, 10]. These variable estrous patterns in cheetahs are not related to season, individual, or age [15, 10]. In addition, estrous signs and behavior are not routinely observed in female cheetahs [15], leading to difficulty in determining estrus for an individual. As a result, ovarian stimulation in cheetahs by exogenous gonadotropins is generally performed without regard to stage of the follicular cycle [10]. Inconsistent ovarian responses after stimulation with exogenous gonadotropins also have been observed in numerous other felid species, such as the domestic cat [5, 16], Asiatic lion [17] and tiger [18]. In cheetahs, poorer ovarian responses and oocyte quality have been documented when exogenous gonadotropins were given during periods of follicular activity [10]. It was reported that when female cheetahs were given equine chorionic gonadotropin (eCG) within 24 h of the estradiol peak (natural estrus, retrospectively determined), significantly higher estradiol excretion was observed, supporting the idea of ovarian hyperstimulation [10]. Further, the morphological quality of oocytes recovered from these females was poor and they failed to complete maturation/undergo fertilization during *in vitro* culture [10].

In the seasonally reproductive domestic cat, the ovary is relatively "quiescent" during the non-breeding season, and estrus induction by exogenous gonadotropin stimulation is more predictable compared to during the breeding season [19]. Further studies in domestic cats confirmed a quiescent ovary more effectively responds to exogenous gonadotropin stimulation with a predictable number of ovulations and a lower incidence of estrogen hypersecretion [20, 21]. Administration of oral progestins (altrenogest, ALT) or progestin implants (levenorgestrel) has been used to improve the responses to ovulation induction with exogenous gonadotropins in domestic cats [22, 23, 16] leading to better assisted reproductive success [20].

Another potential benefit of utilizing exogenous progestin supplementation to induce ovarian quiescence is a reduction in glucocorticoid levels. In other species such as sheep [24] and rats [25], corticosteroids have profound negative effects on mammalian female reproduction.

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Additionally, high fecal glucocorticoid concentrations have been posited as one cause of poor reproductive performance in naturally breeding cheetahs [26]. Acute activation of corticosteroids can modulate hypothalamic GnRH and neuropeptide Y concentrations leading to normal surges of follicle stimulating hormone (FSH) and luteinizing hormone (LH) [27]. However, chronic corticosteroid secretion can inhibit ovarian cyclicity and ovulation [27]. In immature female rats, cortisol exposure impairs the ovarian response to eCG [28]. Cheetah females that received short-term ALT treatment had reduced FGM compared with control animals [10]. Thus, we have speculated that corticosteroid secretion may be related to ovarian hormone responses in female cheetahs.

We have previously shown that follicular activity can be suppressed by progestin treatment, thereby providing a way to quiesce the ovary before gonadotropin treatment [10]. Therefore, the objective of this study was to investigate the follicular and ovulatory responses to exogenous gonadotropin stimulation (eCG and porcine luteinizing hormone; pLH) in captive female cheetahs following short-term pre-treatment with an oral progestin (altrenogest). We hypothesize that the induction of ovarian quiescence by progestin priming before exogenous gonadotropin administration will result in better ovarian responses to eCG/pLH. Ovarian response was assessed laparoscopically to determine number of follicles and corpora lutea, oviduct/uterine cornua diameter and vascularization, and fecal samples were collected for analysis of estradiol (FEM), progesterone (FPM) and glucocorticoid (FGM) metabolite concentrations.

Materials and Methods

Experimental design

Eleven female cheetahs aged 3 to 8 y were included in this study and divided into two groups (one female was included in both treatment groups with a 1-year interval): 1) control, exogenous gonadotropin treatment without ovarian suppression; eCG/pLH (200 IU eCG followed 81-83 h later with 3,000 IU pLH) (n = 5); versus 2) ALT, ovarian suppression prior to gonadotropin treatment; 7 d pre-treatment with ALT (0.1 mg/kg) followed 2 d later by the eCG/pLH protocol (200 IU eCG/ 3000 IU pLH) (n = 7). At 42 h post-pLH, ovarian response was assessed via laparoscopy. Ovarian metrics [number of follicles and corpora lutea, oviduct/uterine cornua diameter (mm), and ovarian vascularization score (0-3)] were recorded. Fecal samples were collected from all cheetahs for analysis of FEM, FPM, FGM by enzyme immunoassay (EIA) as follows: 1) 3-4 samples/wk for 3 wk before ALT treatment (Period 1); 2) daily for 7 d during the ovarian suppression period (Period 2); 3) daily throughout ALT, eCG/LH treatment and laparoscopy (Period 3); and 4) 3-4/wk for 45 d following laparoscopy (Period 4).

Animals

Cheetahs were maintained in four zoological institutions (Smithsonian Conservation Biology Institute (SCBI), VA; White Oak Conservation, FL; Fossil Rim Wildlife Center, TX and Wildlife Safari, OR, USA), all of which are part of the AZA SSP cooperative breeding and

research program. At SCBI, each animal was housed individually and fed a beef-based diet (Natural Balance Pet Foods Inc., Pacoima, CA) 5 d/wk, whole prey (rabbit) 1 d/wk and beef bones (femur, ox tail) 2 d/wk. Each also had *ad libitum* access to water, a permanent barn structure (with heat when ambient temperature was ≤ 40°F) and an unheated yard shelter. At White Oak Conservation, animals were fed a horsemeat-based diet (Milliken Meat Products Ltd, Ontario, Canada) 7 d/wk, and a horse neck bone or venison on bone 1 d/wk. At the Fossil Rim Wildlife Center cheetahs were fed a horsemeat-based diet (Milliken Meat Products Ltd, Ontario, Canada) 6 d/wk, and then either a horse bone, venison on bone, or whole guinea pig or rabbit 1 d/wk. At Wildlife Safari, cheetahs received horse meat on bone and organ meat 6 d/week with Nebraska Slabmeat Supplement powder (Central Nebraska Packing, Inc., North Platte, NE) added, and one gutted and skinned rabbit per wk, as possible. All animal-related procedures were approved by the National Zoological Park's Institutional Animal Care and Use Committee (IACUC) and similar committees at each of the collaborating institutions.

Ovarian suppression, estrus/ovulation induction and ovarian/reproductive tract assessment

Altrenogest oral suspension (2.2 mg/mL Regumate; Intervet Inc., Millsboro, DE, USA) was stored at room temperature in an opaque container [16]. During the period of ALT dosing, 0.1 mg/kg ALT was mixed into each cheetah's food daily for seven consecutive days. For follicular growth and maturation, female cheetahs were treated intramuscularly with a single administration of 200 IU eCG (Sigma Chemical Co., St. Louis, MO) two days after the ALT treatment ended. The intramuscular administration of 3000 IU pLH (Sioux Pharm, Sioux City, IA, USA) was performed to induce ovulation 81-83 h later.

Post-pLH administration for 42 h, ovarian activity was assessed by laparoscopy. Females were anesthetized using a combination of ketamine hydrochloride (2.0–3.5 mg/kg body weight; Ketaset, Zoetis, Parsippany, NJ, USA) and medetomidine hydrochloride (22–25 µg/kg; Domitor, Zoetis), or a combination of the latter drug (40 µg/kg), butorphanol tartrate (0.3 mg/kg; Torbugesic-SA, Zoetis) and midazolam (0.2 mg/kg; Midazolam, Pfizer, NY, USA), all administered intramuscularly [29]. After tracheal intubation, surgical anesthesia was maintained with isoflurane. Laparoscopy was performed as described by Crosier et al. [29]. Briefly, the females were placed in ventral recumbency and in a head-down position. Following insufflation, laparoscopy was performed by insertion of 5-mm diameter laparoscope (Olympus Corporation, Lake Success, NY, USA) on the abdominal midline. All aspects of each ovary were classified as: 1) presence of mature follicles (diameter ≥ 2 mm) or post-ovulatory corpora luteal (CL) (opaque, reddish-yellowish structures raised above ovarian surface); or 2) absence of either follicles or corpora lutea. All ovarian metrics (number and size of follicles and corpora lutea on each ovary), ovarian and uterine vascularization score (0-3, where 0 = no vascularization and 3 = extremely vascular) were recorded. In addition, the size (mm) of each oviduct and uterine cornua were precisely measured by transabdominal insertion of a graduated Verres probe [4, 29].

Fecal collection and extraction

Fecal samples were collected at a frequency of 3-4 samples/wk during Period 1 and Period 4, and daily in Periods 2 and 3. Samples were collected within 24 h of defecation, sealed in individual sterile plastic bags, labeled with the animal name/date, and stored frozen (-20°C) until freeze-dried (Lyophilizer; Labconco, Kansas City, MO). After drying, samples were pulverized with a rubber mallet and stored in labeled, plastic tubes at -20°C until further processing. A volume of 0.1 mL of progesterone ^{3}H tracer (~6,000 dpm) was added to each sample and used to determine extraction efficiency. A total of 5 mL 90% ethanol was added to each dry fecal sample (0.2 ± 0.2 g) and shaken for 20 min. Samples were centrifuged (1500 x g for 20 min), the supernatant was recovered into a new tube and the fecal pellet was resuspended in 5 mL 90% ethanol, vortexed for 30 sec and centrifuged (1500 x g for 15 min). The first and second supernatants were combined, dried under air for 1-2 d and reconstituted in 1 mL 100% methanol. The methanol extract was vortexed briefly and placed in a sonicator for 15 min to free particles adhering to the glass tube, dried again under air for 3-6 h, and resuspended with 1 mL dilution buffer (5.4 g NaH$_2$PO$_4$ (Sigma-Aldrich, St. Louis, MO, USA), 8.66 g Na$_2$HPO$_4$ (Sigma-Aldrich), 8.7 g NaCl (Sigma-Aldrich) in 1000 mL Milli-Q water). The extracted fecal samples were briefly sonicated and stored in polypropylene tubes at -20°C until EIA analyses. Extracted fecal sample efficiency was evaluated with a beta counter (LS 6500 Multi-Purpose Scintillation Counter, Beckman Coulter, Pasadena, CA, USA).

Hormonal metabolite assessments

FEM and FPM [29] and FGM [30] concentrations were measured by single-antibody EIAs validated for cheetahs, as described previously. Antibodies (polyclonal estradiol, R4972, 1:50,000 dilution; monoclonal progesterone, CL425, 1;10,000; polyclonal cortisol, R4866, 1:8,500) and horseradish peroxidase-labeled tracers (estradiol, 1:50,000; progesterone, 1:40,000; cortisol, 1:20,000) were provided by Coralie Munro (University of California, Davis). Intra-assay coefficients of variation were < 10% for all EIAs, and inter-assay variation for two internal controls were 12.7% and 14.9%, 13.9% and 15.5%, and 12.1% and 15.8% at ~ 30% and 70% binding for the estradiol, progesterone and glucocorticoid EIAs, respectively. All fecal hormone concentrations are expressed as µg/g dry feces.

Statistical analysis

Baseline concentrations of each hormone for each female were calculated using an iterative process whereby concentrations above the mean + 1.5 (FEM, FGM) or 2 (FPM) SD were removed and the values recalculated until no points above those values remained [31]. Elevated concentrations of FEM [29] and FGM [10] were those 1.5 times above the hormonal baseline, whereas 3 times the baseline for 5 consecutive days was considered elevated for FPM. An estradiol peak was considered for a sample elevated 1.5x above the hormonal baseline value. Hormone data were averaged (mean ± SE) within the four time periods for each female (See Experimental Design), and those means averaged for analysis.

Data analysis was performed using SAS (SAS Institute Inc., 2002, Cary, NC, USA, version 9). Normal distribution of residuals from the statistical models was tested using the UNIVARIATE procedure option NORMAL. The statistical models included the fixed effect of time (period). The dependent variables (ovarian metrics, reproductive tract metrics) and average concentration of fecal steroid hormonal were analyzed by ANOVA (GLM procedure). Differences in ovarian metrics, reproductive tract diameter and fecal steroid hormonal concentration between control and ALT groups among the four time periods were compared using Paired t-test when the data were distributed normally and the Wilcoxon signed rank test when the data were not distributed normally. Differences in fecal steroid hormonal average concentrations between treatment groups were analyzed using repeated measurement mixed model ANOVA. The ovarian, oviduct and uterine body vascularization score (0 to 3) was compared using NPAR1WAY procedure and a Kruskall-Wallis test. The number of hormonal peaks was analyzed by a binomial score (1 or 0) based on the presence or absence of a peak in each period (Period 1-4). The level of significance was set at $P \leq 0.05$.

Results

Ovarian metrics and reproductive tract appearance

Mature follicles (diameter ≥ 2 mm) and/or fresh corpora lutea were observed in all cheetahs, and numbers did not differ between control and ALT-treated females ($P > 0.05$; Table 1). The number of mature follicles in the control and ALT groups ranged from 0-17 and 4-7, respectively. The number of corpora lutea ranged from 0-9 in both groups. Similar to ovarian metrics, diameter of both oviducts and uterine cornua (left and right) did not differ between groups ($P > 0.05$), except for the left uterine cornua diameter, which was larger for ALT females than for the control group ($P \leq 0.05$; Table 1). The vascularization scores of the ovary, oviduct and uterine cornua were not different between ALT or control females ($P > 0.05$).

The influence of ovarian suppression on fecal progesterone metabolites

Within time periods of sample collection, FPM average concentrations were similar between control and ALT groups ($P > 0.05$), except during ALT treatment (Period 2) when FPM average concentration was higher for the females receiving altrenogest ($P \leq 0.05$; Table 2). However, within both groups, FPM average concentrations were similar throughout Periods 1-3, and then increased ($P \leq 0.05$) approximately 10-fold in response to gonadotropin stimulation (Period 4 for both groups; Table 2). The average length of the non-pregnant luteal phase did not differ ($P > 0.05$) between groups (control: 33.6 ± 10.6 d vs ALT: 36.0 ± 10.7 d). Interestingly, during Period 4, only two of the five cheetahs (40%) in the control group produced consistently elevated FPM concentrations of 3-times baseline levels during the non-pregnant luteal phase for the 45-d period after laparoscopy. Of the other three, one produced elevated FPM concentrations for only 38 days following laparoscopy, one produced elevated concentrations for 43 days after laparoscopy, and the third female did not produce elevated FPM concentrations until 8 days after laparoscopy, which then remained elevated throughout the rest of her non-pregnant luteal phase.

For the ALT group, 4 of 7 cheetahs (57.1%) produced FGM values that were at least 3-times baseline levels for the entire 45-d period following laparoscopy. Of the three females that did not, one did not produce elevated FPM concentrations until 8 days after laparoscopy, and then remained elevated throughout the rest of her non-pregnant luteal phase, one female produced consistently elevated FPM concentrations for 43 days following laparoscopy; and the third female produced elevated FPM concentrations for 37 of the 45 days post laparoscopy.

The influence of ovarian suppression on fecal estradiol metabolites

In the control group, FEM average concentrations remained relatively constant across all periods of sample collection (Table 2). Comparatively, FEM average concentrations for females in the ALT group were elevated in Period 3 compared to the values for both Periods 1 and 2 ($P \leq$ 0.05). These elevated FEM concentrations were then maintained for females in the ALT group during Period 4 (Table 2). In the control group, 2 of 5 females appeared to be cycling based on the presence of FEM peaks before ovulation induction during Periods 1 and 2. In the ALT group, 3 of 7 females were cycling, but did not exhibit further FEM peaks after oral administration commenced. After eCG, FEM peaks were observed in four of five control females (80%) and all of the ALT-treated females (7 of 7) (Period 3; range 2-4 d after eCG treatment in both groups; Fig. 1). Furthermore, the highest FEM peak concentration after eCG administration (Period 3) in the ALT group was 1.46 µg/g dry feces (range: 0.31-1.46 µg/g dry feces), whereas the highest peak of FEM concentration was 0.74 µg/g dry feces (range: 0.29-0.74 µg/g dry feces) for control females.

The influence of ovarian suppression on fecal glucocorticoid metabolites

For control females, the FGM average concentration did not vary among periods of sample collection (Table 2). By comparison, FGM average concentrations for females in the ALT group decreased ($P \leq 0.05$) in Period 2 compared to Period 1, and remained low during Period 3 (Table 2). Additionally, the control females had higher ($P \leq 0.05$) FGM average concentration during Period 2 compared to females in the ALT group during this same time period (Table 2).

The number of FGM peaks observed during Periods 2 and 3 in the control group was higher ($P < 0.05$) than the number of peaks for females in the ALT group during these same periods. FGM peaks (1-4 peaks/individual) were observed in 3 of 5 control group cheetahs (60%) during Periods 2 and 3 (Fig. 2A, 2B). Comparatively, FGM peaks (1-4 peaks/individual) in the ALT group were observed in 3 of 7 (42.9%) and 2 of 7 (28.5%) female cheetahs in Periods 2 and 3, respectively. The number of PGM peaks did not differ ($P > 0.05$) between groups during Period 1, where the control females had 1-4 peaks/individual compared with the same (1-4 peaks/individual) for the ALT group. Similarly, during Period 4, control females produced 3-9 peaks/individual, which was not different ($P > 0.05$) than the range for the ALT females of 2-10 peaks/individual.

Discussion

The present study demonstrated that ovarian responsiveness to eCG/pLH in the female cheetah was improved after short-term oral progestin administration. This was confirmed in two ways. First, there was the expected elevation in FEM average concentrations following eCG administration in ALT-treated females; however, hyperestrogenism did not occur prior to estrus induction when compared to controls. In addition, short-term exogenous progestin supplementation reduced FGM concentrations during the gonadotropin treatment period, which may have contributed to the improved estradiol production patterns observed.

Earlier studies in domestic cats demonstrated the efficacy of exogenous progestins which, when administered short-term, rapidly and reversibly inhibited follicular activity and positively altered sensitivity to exogenous gonadotropins during estrus/ovulation induction [23, 32, 16]. Species-specific physiology as well as individual metrics, such as stage of the estrous cycle when treatment commences, can influence the success of ovarian suppression by exogenous gonadotropins. The present study demonstrated increased concentrations of FEM after eCG administration in female cheetahs that were pre-treated for 7 d with ALT, and confirmed a previous study that hyper-estrogen excretion in domestic cats was mitigated by preemptive ovarian down-regulation with 38 d of oral progestins [33]. We observed similar results in this study, with the treated females producing reduced estradiol levels during the ALT administration, followed by an increase in estradiol due to the exogenous hormone stimulation. This reduction of estradiol production during the treatment period is a positive result of the ALT administration, demonstrating a more quiescent ovary at the time of eCG/LH stimulation.

Oral progestins down regulate ovarian function through a negative feedback pathway by binding the progesterone receptor of target cells in the hypothalamus and inhibiting release of LH from the pituitary gland [34]. This down regulation leads to a more efficient and rapid response to miniscule amounts of exogenous gonadotropins by increasing overall sensitivity of the ovary to stimulation [33]. Apart from this down regulation effect via progesterone receptor binding, oral progestins may also work synergistically with the direct effects of steroidogenesis and granulosa cell remodeling at the level of ovary [32, 16], leading to the down regulation of follicle development during the time of treatment. Moreover, exogenous progestins could persistently block pre-ovulatory LH surges and generate constant and low LH levels throughout the entire ovarian suppression period [35]. There also is evidence that exogenous progesterone exposure is related to the steroidogenesis and receptor population alteration in the hypothalamic pituitary-gonadal axis, which is turn causes down regulation of ovarian function [36]. It has been reported in the domestic cat that the positive effect of exogenous progesterone priming on the consistency of ovarian progesterone production may be related more to the ability of the progestin to suppress ovarian activity [33, 37]. The quiescent ovary after ovarian suppression could support the normally steroidogenic function in response to the proper exogenous gonadotropin dosages [33, 37]. Previous research has established that 3β-hydroxysteroid dehydrogenase (3β-HSD) gene expression was involved with progesterone biosynthesis [38, 39]. In domestic cats, 3β-HSD gene expression was decreased by pre-emptive oral progestin

compared to the non-ALT treatment group [37]. In contrast, the up regulation of 3β-HSD gene was found in unprimed ALT females [37] leading to the increase of progesterone biosynthesis [38, 39]. This phenomenon could be altering the ovarian sensitivity and ovarian response after exogenous gonadotropin exposure [37]. Thus, this evidence supports our finding that the consistent non-pregnant luteal phase observed in females receiving the ALT treatment may be due to the positive effect of exogenous progesterone priming. However, additional research, including on gene regulation associated with the progesterone biosynthesis pathway in female cheetahs, would be necessary to further to confirm this hypothesis.

FPM increased during Period 4 (after pLH treatment) in both groups, with concentrations differing between control and treatment groups during Period 2. There were more females with a consistent FPM elevation over baseline (non-pregnant luteal phase) for 45 d following laparoscopy in the ovarian-suppressed group compared with control females, suggesting better ovarian responses and corpora lutea function in females receiving oral progestin therapy. Thus, our data support the supposition that normalization of progesterone production following oral progestin and exogenous gonadotropins can improve follicular development when stimulated during a quiescent period, subsequently resulting in more healthy and productive corpora lutea [33]. A concern upon commencement of this research was that prolonged progestin exposure used in domestic cats [33, 37] (i.e., 38 d) might negatively affect subsequent normal pituitary or ovarian function. Based on our previous research, the cheetah female demonstrates short and inconsistent estradiol peaks [10], so a shorter treatment period of 7 d was used in this study. Other studies of ALT function in various species support the efficacy of shorter-term (7 to 14 d) oral progestin protocols to induce a quiescent ovary and synchronize resumption of follicular growth following the end of progestin treatment [40, 41].

A decrease in FGM was also observed in cheetahs during altrenogest treatment. Adrenal activity in women was similarly suppressed by treatment with a high dose of the exogenous progesterone, medoxyprogesterone acetate [42]. Also, in a study of adult female rats, it was demonstrated that chronic exogenous progesterone administration (21 d) lowered adrenal/body mass ratios and reduced serum corticosterone [43]. Progesterone has a high affinity for mineralocorticoid receptors [44] and for glucocorticoid receptors [45], which may reduce its bioavailability, although this has not been proven in cheetahs. Interactions between progesterone and glucocorticoids might be one cause of the negative feedback on the hypothalamic-pituitary-adrenal axis and may lead to the reduction of adrenal cortisol activity and glucocorticoid synthesis [43].

An interesting finding was that uterine horn diameters in the ALT treatment group were significantly larger than those in the control group, which is in contrast with studies in dogs and horses [46, 47]. The study in dogs noted haemodynamic changes in the uterine artery and uterine diameter during proestrus, while uterine blood flow diminished during estrus and anestrus [46]. In the mare, the presence of a preovulatory follicle or corpora lutea had no differential effects on uterine horn diameter [47]. By contrast, cattle after superovulation with exogenous gonadotropins (eCG) presented with higher uterine gland density compared to the control group

[48]. Increased progesterone secretion in response to eCG may play a role in cell proliferation, differentiation, and gland development in the uterine environment [48]. In our study, ovarian responses, including progesterone production, to eCG were greater in the ALT treatment group controls, which might explain the increase in uterine diameter in that group.

The findings of our present study support the advantages of exogenous progestin administration to generate a more uniform ovarian response in follicular development and ovulation induction, which was previously reported in both domestic and non-domestic cat species [33, 37, 10]. Our results suggest that ovarian response to exogenous gonadotropin treatment (eCG/pLH) in the female cheetah is improved after short-term oral progestin (0.1 mg/kg ALT) administration. Females pre-treated with ALT exhibited consistent increases in FEM after eCG as indicated by higher peaks over baseline levels (without hyperestrogenism). A more homogeneous non-pregnant luteal phase as measured by consistent FPM levels was observed in ALT treated female cheetahs. Additionally, FGM were decreased during the time of ALT treatment, which may increase the efficacy of exogenous gonadotropins. In summary, short-term ovarian suppression using oral progestin may be a practical tool for improving ovulation induction in the cheetah.

Acknowledgements

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