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Noninvasive pregnancy detection in wild felids using Enzyme Immunoassay of fecal 13, 14–dihydro–15–keto–$PGF_2\alpha$ metabolite (PGFM)

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Abstract: The study was carried out on Panthera species (4 lions and 5 tigers) housed in Bannerghatta Biological Park, Bengaluru, and Sri Chamarajendra Zoological Gardens, Mysuru. Estrus behavior such as rolling, licking, nudging, chuffing, frequent urination, whitish discharge, vocalisation, lordosis and copulatory behavior such as vocalisation, allowed to be mounted, aggression towards male, mounting, and biting of the nape of female were exhibited both in lions and tigers which helped to identify the time of fecal sample collection. The study's main aim was to carry out noninvasive and noncontact pregnancy detection in these wild felids. Feces samples collected from the female animals on day 0 (day of mating), 30, 45, 60, 90, 100, and 10th day postpartum were analysed for 13, 14–dihydro–15–keto–$PGF_2\alpha$ metabolite (PGFM) levels using Enzyme Immunoassay (EIA). A significant increase in PGFM concentrations was seen from day 45 to day 60 in pregnant lionesses and tigresses used to detect pregnancy. The PGFM levels from day 60 were significantly different between pregnant and nonpregnant lionesses and tigresses. The PGFM levels were found to rise from day 45 and drop to basal levels by day 60 in 2 tigresses which may be due to fetal resorption. This study effectively detected pregnancy in lionesses and tigresses between day 45 and day 60 but requires further investigation due to the paucity in literature about PGFM analysis in tigers and lions.

Key words: Lioness, tigress, pregnancy, $PGF_2\alpha$ metabolite, enzyme immunoassay

1. Introduction

The felidae family has 40 species, most of which are classified by International Union for Conservation of Nature (IUCN)[1] as threatened, vulnerable or endangered because of illegal hunting, habitat loss and degradation, except the domestic cat. Ex-situ conservation programmes have been implemented to stabilise the factors endangering wild felines in their natural habitat [1]. In the early 90s, the success of captive breeding for threatened species became one of the more popular methods for saving endangered species, but it depended on a better understanding of the reproductive capacity (fertility status, time of ovulation, optimum time for mating, pregnancy, etc.) of animals [1]. The Panthera lineage includes some of the important endangered species like tigers (Panthera tigris), lions (Panthera leo), jaguars (Panthera onca), and leopards (Panthera pardus) [2].

There are various methods for pregnancy diagnosis like hormone estimation by blood sample collection and ultrasonography which bears more risk of stress and abortion. Therefore, noninvasive procedures have been established to assess the pregnancy status using feces and urine samples in various species using endocrine analysis [3]. Monitoring of pregnancy status using feces samples provides an important and noncontact alternative for pregnancy diagnosis. Also, it helps to assess reproductive activity in felines compared with traditional invasive methods.

1.1. Pregnancy diagnosis in wild felids

The substantial risk of abortion brought on by stress and/or anesthesia prevents frequent blood sampling in wild animals for reproductive hormone study. Therefore, it is preferred to diagnose pregnancy by taking a urine or fecal sample to avoid such situations. The noninvasive techniques for urinary and fecal steroid analysis (estrogen and progesterone) that have been developed over the past

[1]IUCN (2022). The IUCN Red List of Threatened Species. Version 2022-1 [Online]. Website https://www.iucnredlist.org [Accessed 21st November 2022].

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few decades are used for pregnancy diagnosis in several species of felines [1]. Still, Bergfelt et al. [4] showed that the placental hormone $PGF_2\alpha$ metabolite (PGFM) can be analysed for pregnancy detection instead of steroids. Schramm et al. [5] reported a false increase of fecal progesterone in lionesses due to spontaneous ovulation in the absence of mating and hence failed to detect pregnancy accurately. Previous results from several wild felid species indicated that PGFM differentiates between pregnancy and pseudo-pregnancy in captive and free-ranging felids, whereas progesterone does not [6]. Only pregnant cats show an elevation in PGFM levels which indicates the placental origin of this hormone and hence accurate pregnancy diagnosis [7].

1.2. PGFM and its metabolism

The detection of PGFM in feces proved to be a fast and nonstressful tool for pregnancy diagnosis for various captive and free-ranging animal species [8]. The level of PGFM starts to rise by day 30 postmating and peaks from day 45 till parturition [6,9]. Pregnancy and estrous cycle-related serum PGFM profiles have been described before in several species such as bitch, buffalo [10], rabbit [11], and domestic cat [12]. $PGF_2\alpha$ secreted from the uterus and placenta is involved in the regulation of reproductive processes such as early embryonic development, initiation of parturition, and resumption of postpartum ovarian activity in canines, felines, and humans [6]. $PGF_2\alpha$ metabolite PGFM is an indicator of pregnancy when determined by Liquid Chromatography-Mass Spectrometry (LCMS), High Pressure Liquid Chromatography (HPLC), and Enzyme Immno-Assay (EIA) of fecal and urine samples [6,13]. Increasing PGFM concentration in fecal samples is enough to detect pregnancy, without knowledge of the breeding date, because a clear demarcation between pregnant and pseudo-pregnant females is noted when PGFM spikes suddenly around day 30–45 [7]. $PGF_2\alpha$ is metabolised to PGFM (13, 14–dihydro–15–keto–$PGF_2\alpha$) during the first passage through the lungs [14]. The hormone is rapidly metabolised in the liver to its plasma metabolite (PGFM, 13, 14–dihydro–15–keto– $PGF_2\alpha$) which is excreted in urine and feces [6]. Measurable concentrations of PGFM are said to be stable in feces stored at –20 °C for up to 2 years till further analysis [13].

To detect pregnancy status in captive wild felids which have been bred, the present study was designed with the following objectives: Detection of pregnancy in wild felids (tiger and lion) using EIA of fecal 13, 14 –dihydro–15–keto–$PGF_2\alpha$ metabolite (PGFM).

2. Materials and methods

Bannerghatta Biological Park, Bengaluru (12.9716 °N, 77.5946 °E) and Chamarajendra Zoological Garden, Mysuru (12.2958 °N, 76.6394 °E) is situated in the state of Karnataka. The study was carried out between March 2022 and January 2023 with due permission of the Principal Chief Conservator of Forests (Wildlife) and Chief Wildlife Warden, Bengaluru vide No. PCCF(WL)/E2/MISC/CR-05/2020-2021 dated 07/03/2022.

2.1. Selection of animals

Data was collected on a total of 9 individuals representing two species. They included tigers (Panthera tigris) and lions (Panthera leo) housed in Bannerghatta Biological Park, Bengaluru, and Chamarajendra Zoological Garden, Mysuru. The animals are managed and housed according to the rules and regulations set by the Zoo Authority of Karnataka. The animals are provided with adequate outdoor and indoor spaces with small water bodies and ample shade for the summer heat. The sexually active male and female lions and tigers were housed together when the female was in estrus and kept in separate enclosures otherwise. In some animals, the mating pairs develop familiarity and hence are kept in the same enclosure. The pregnant females are separated from males before parturition and the cubs are housed with the mother at least for 6 months. Male and female tigers and lions in both zoos are fed 6 days a week, males consume 9–10 kg/day of meat, and females consume 8–9 kg/day of meat. The diet mainly consists of locally sourced and vetted beef and chicken. Animals are provided with ad libitum water in the enclosure, kraals as well as the display area. Female animals above 4 years of age, showing estrus signs, and left to breed with males were considered for the study and collection of fecal samples.

2.2. Fecal sample collection and storage

Approximately 50 g of fresh fecal sample was collected using sterile 100 mL plastic containers from female tigers and lions that expressed signs of heat and bred in that estrous cycle. Fecal samples were collected from females that have been bred on day 0 (day of mating), 30, 45, 60, 90, 100, and 10^{th} day postpartum. Fecal samples were collected in the morning hours before 9:00 am and immediately stored at –20 °C until further analysis. If transportation from the collection/storage site to the place of analysis was necessary, it was done using a 4 °C ice container within 2 to 3 h.

2.3. Fecal sample extraction

Fecal samples were subjected to an extraction procedure as follows:

1. Fecal samples were dried in a hot air oven at 60 °C.
2. 0.5 g of dried fecal sample was extracted with 5 mL 100% ethanol by shaking for 30 min at 650 rpm in a digital vortex mixer (Digital Vortex Mixer: iSwix VT obtained from Neuation Technologies Pvt. Ltd., Gandhinagar, Gujarat, India).

3. The vortexed sample was centrifuged for 20 min at 2500 rpm. (REMI R-8C Centrifuge machine from REMI Lab World, Mumbai, Maharashtra, India)
4. The supernatant was reserved in sterile tubes and stored at –20 °C until further analysis.
5. Aliquots of fecal extracts were then used to detect the concentration of PGFM using EIA.

2.4. Determination of PGFM

The DetectX® 13, 14–dihydro–15–keto–$PGF_2\alpha$ (PGFM) Immunoassay Kit by Arbor Assays, Michigan, USA was used to determine PGFM concentration for the fecal extracts. For immunoassay, the ethanol content in the assay typically must be ≤5 %, and hence the extract was additionally diluted using Assay Buffer. 25 µL of fecal extract was diluted with 475 µL of Assay Buffer (1:20) making the final ethanol concentration of 5 %.

The 4 Parameter Logistic (4PL) Curve software capabilities that were specific to the DetectX® PGFM Kit were used to calculate PGFM concentration for each unknown sample after obtaining optical density generated at 450 nm.

2.4 Statistical analysis

The PGFM concentration on Days 0, 30, 45, 60, 90, 100, and 10th days postpartum for pregnant animals analysed using Enzyme Immunoassay (EIA) were subjected to repeat measures ANOVA and post hoc Tukey's multiple comparison test to check significance between the days and Paired t-test was used to analyse the significance between day 0 and other days. Comparison for significant differences between pregnant and nonpregnant animals or pregnant and animals which have undergone resorption were done using an Independent Sample t-test. All statistical analysis was done using SPSS (Statistical Package for Social Sciences) statistics software, Version 16.0.

3. Results

Estrus behavior *viz.* rolling, licking, nudging, chuffing, frequent urination, whitish discharge, vocalization, lordosis, and copulatory behavior such as vocalisation, allowed to be mounted, aggression towards male, mounting, and biting of the nape of female was exhibited both in lions and tigers. Out of 4 lionesses, 3 were pregnant and out of 5 tigresses, 2 were found to be pregnant. Two of the tigresses in the study were suspected to be a case of fetal resorption. The PGFM concentrations measured from fecal extracts of the study animals yielded the following results.

3.1. PGFM concentrations in pregnant lioness and tigress

On day 60, there was a significant rise ($p < 0.05$) in the PGFM concentration when compared to day 45 in pregnant lions and tigers of this study. This significant change in the concentration from day 45 to day 60 was used as an indicator of positive pregnancy status in the case of lions. In this study, the mean PGFM concentration of the 10th day postpartum fecal sample was found to return to its basal level which was statistically significant ($p < 0.05$) and lower when compared to days 60, 90, and 100 of gestation as shown in Table 1 and 2.

3.2. PGFM concentrations between pregnant and non-pregnant lioness and tigress

In our study, mean PGFM levels on day 60 of pregnant lionesses (3279.17 ± 195.72 ng/g of feces) was significantly higher when compared to nonpregnant lionesses (475.5 ng/g of feces) ($p = 0.019$) as shown in Figure 1. There was also a significant difference in mean PGFM levels between pregnant and nonpregnant tigresses on day 60 (4900.00 ± 483.00 ng/g of feces v/s 910.00 ± 354.91 ng/g of feces, $p = 0.006$) as shown in Figure 2. Hence this statistical difference in concentration of PGFM on day 60 could be considered as a deciding factor for the pregnancy status of a lioness and tigresses.

In the present study, there was a significant statistical difference between tigresses that were pregnant and may have undergone fetal resorption on day 60 (4900.00 ± 483.00 v/s 1179.00 ± 401.00 ng/g of feces, $p = 0.027$), day 90 (6482.00 ± 56.00 v/s 749.50 ± 173.50 ng/g of feces, $p = 0.001$) and day 100 (7628.50 ± 397.50 v/s 807.90 ± 217.10 ng/g of feces, $p = 0.004$) (Figure 3). The pattern of rise in PGFM levels from day 0 to day 45 and drop to basal levels by day 60 of gestation in 2 of the tigresses could be due to fetal resorption.

4. Discussion

According to Siemieniuch et al. [15], the PGFM level in feline plasma was low during the first and second trimesters of pregnancy before beginning to rise during the final three weeks of pregnancy. In wild felids like sand cats, fishing cats, cheetahs, lynxes, ocelots, oncillas, caracals, and serval, PGFM levels divert from basal concentrations solely during the last few weeks of pregnancy depending on their gestation length [13]. Kustritz [16] reports that pregnancy in the last trimester is possibly maintained by uterine sources of progesterone and other hormones like prolactin. Hence, CL undergoes luteolysis via $PGF_2\alpha$ produced by the fetoplacental unit which in turn increases the plasma PGFM concentrations [17].

4.1. PGFM concentrations in pregnant lioness and tigress

In line with our study, basal concentrations of PGFM (830–1100 ng/g of feces) in Asiatic lions, started to increase from 40 to 60 days onwards till 80 to 100 days and a sharp rise a week before parturition (4987 ± 615 ng/g of feces) [9]. Compared to earlier reports, in the Panthera lineage, e.g., the black panther, the Sumatran tiger, and the Chinese leopard had higher levels of PGFM during the last 10 to 20 days of gestation [13]. The overall PGFM levels in our study were higher than in previous reports [9,13].

This variation in PGFM concentrations may be due to the difference in the EIA kits used, species difference or individual animal variation [9]. Pregnant female lynxes' PGFM levels increase from day 35 post-mating itself and reach a peak of about 2700 ng/g around parturition at days 61 to 65, and then return to baseline following delivery [6]. This increase in the PGFM levels from the start of the second trimester of gestation was similar to the rising trend of PGFM concentration in our study.

Dehnhard et al. [9] observed that PGFM levels ranged from 1133 to 1665 ng/g throughout the first five weeks (0 to 30 days) of pregnancy in four pregnant Bengal tigers after which it dramatically increased, and continued to rise for the remainder of the pregnancy, peaked the week before delivery (6650 ± 623.8 ng/g of feces) and returned to basal levels (1500 ng/g of feces) 7 to 10 days after delivery. This rise in PGFM at around 40 to 60 days and peak concentrations at around 100 days was similar to our results. Dehnhard et al. [13] said the maximum levels of PGFM were found to be 400 and 2000 ng/g of feces in pregnant Indochinese and Sumatran tigers respectively which is much lower than the individual concentrations obtained in our study. The concentrations of PGFM were found to be inconsistent due to individual animal variation and differences in the EIA kits used.

In domestic cat, leopard cat, and lynx lineages the PGFM levels were seen to increase dramatically from day 30 to 40 depending on the gestation length of the individual species, which was used to confirm the pregnancy status of the animal. In contrast, the animals of panther lineage showed a distinct increase in PGFM levels on day 75 (Chinese leopard and Sumatran tiger) and day 82 (Black panther) which was in concurrence with our results where the significant increase was noted between day 45 and day 90 [13].

4.2. PGFM concentrations in pregnant and nonpregnant lioness and tigress

Our observations were in accordance with Dehnhard et al. [9] who stated that the basal levels of PGFM during the first 5 weeks (1133 to 1665 ng/g of feces), increased significantly at the 9th week (> 2000 ng/g of feces), stayed elevated through the course of gestation and showed peak levels few weeks before parturition (6650 ± 623.8 ng/g of feces). PGFM levels were compared between the terms and showed a significant rise from the first (1 to 5 weeks) to the last term (11 to 15 weeks) which are comparable to the increase in PGFM levels of the tigresses in our study. There was a large PGFM rise above baseline 3 to 4 weeks before parturition and significantly peaks from 45 days (start of the final trimester) till parturition in most pregnant felid species compared to nonpregnant animals of the same species [6,9,13].

4.3. PGFM concentration in tigresses with fetal resorption

The profiles of the two female lynx provided evidence for this since preterm pregnancy termination was followed by an immediate drop in PGFM. The sharp decline in fecal PGFM concentrations at the time of abortion and premature birth further suggests a close association between this hormone and the maintenance of a full-term pregnancy [13]. The length of pseudo-pregnancy in the domestic cat were reported to range from 32 to 56 days [18] and 40 to 50 days [19]. Whereas, Graham et al. [20] stated that pseudo-pregnancy lasted 55 days in lions based on sustained levels of fecal progesterone. The ability of PGFM to remain at baseline without any increases in case of pseudo-pregnancy, allowed cat breeders to distinguish between pregnant and pseudo-pregnant queens. However, in most felid species (domestic cat, sand cat, rusty-spotted cat, fishing cat, Iberian lynx, oncilla, ocelot, caracal, and

Table 1. PGFM concentration (ng/g) in pregnant lionesses (mean ± SE, n = 3).

Day	**PGFM concentration (ng/g)**
Day 0	376.13 ± 53.41^{a}
Day 30	481.60 ± 26.73^{a}
Day 45	1170.93 ± 352.24^{a}
Day 60	3279.17 ± 195.72^{b}
Day 90	6244.60 ± 164.76^{c}
Day 100	7847.67 ± 258.73^{d}
10th day post-partum	332.57 ± 43.31^{a}

Note: The values bearing different superscripts (a, b, c, and d) within the column vary significantly ($p < 0.05$)

Table 2. PGFM concentration (ng/g) in pregnant tigresses (mean ± SE, n = 2).

Day	PGFM concentration (ng/g)
Day 0	934.60 ± 145.40[a]
Day 30	1336.40 ± 302.40[a]
Day 45	1668.00 ± 377.00[a]
Day 60	4900.00 ± 483.00[b]
Day 90	6482.00 ± 56.00[bc]
Day 100	7628.50 ± 397.50[c]
10th day postpartum	746.75 ± 111.35[a]

Note: The values bearing different superscripts (a, b, c) within the column vary significantly ($p < 0.05$).

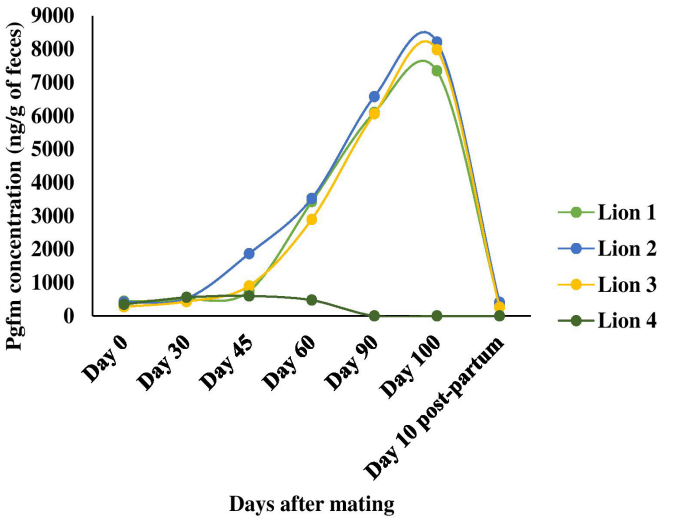

Figure 1. The concentration of PGFM (ng/g) in pregnant (Lion 1, 2, and 3) and nonpregnant lions (Lion 4).

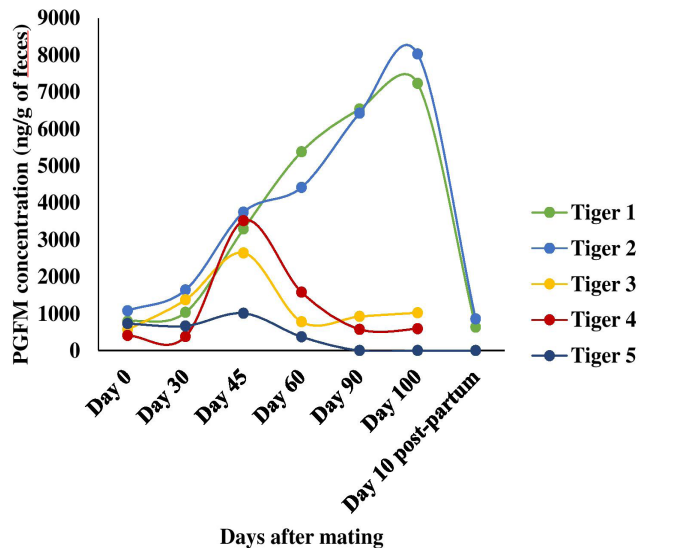

Figure 2. Concentration of PGFM (ng/g) in pregnant (Tiger 1 and 2) and nonpregnant tigers (Tiger 3, 4, and 5).

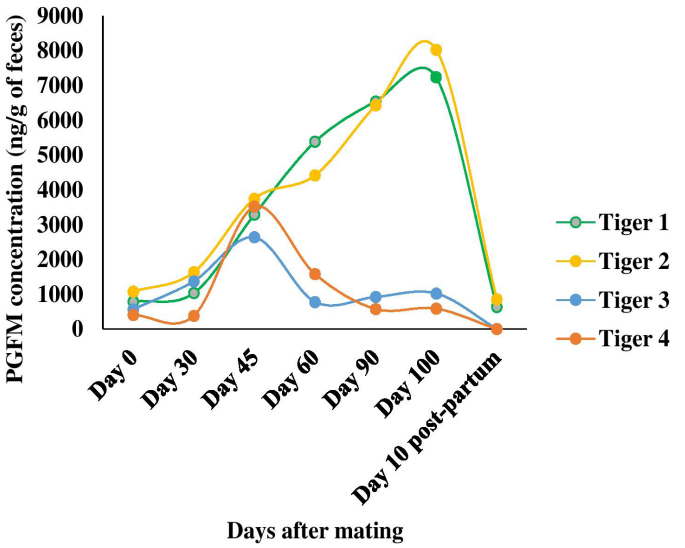

Figure 3. Concentration of PGFM (ng/g) in tigers which are pregnant (Tiger 1 and 2) and have undergone fetal resorption (Tiger 3 and 4).

Sumatran tiger) there was no elevation of PGFM above baseline in pseudo-pregnant females. In contrast, elevated levels were observed during the last trimester of pregnant animals [7,13].

4. Conclusion

Measuring the concentration of PGFM for pregnancy detection is a new and not yet fully explored territory. Since blood collection and ultrasonography used to diagnose pregnancy bears a higher risk in wild felines due to stress of handling and general anesthesia if required, fecal sample collection has become one of the methods that has been recently exploited. In the present study, PGFM levels measured from fecal samples using EIA in pregnant and nonpregnant lionesses and tigresses have helped us effectively detect pregnancy in these animals.

In this study, PGFM concentrations of pregnant animals were seen to rise significantly between day 45 and 60, which was used to detect pregnancy accurately. When the PGFM rises above 6000 ng/g of feces the lioness or tigress is delivered in the next 10 to 15 days and this type of prediction of parturition helps coordinate zoo practices to provide better care during the process of parturition and newborn care by which we can reduce cub mortality. Resources for the management of pregnant captive wild felids can be done in a better way to ensure a successful captive breeding programme. PGFM levels provide a clear picture of pregnancy status, but, this method of pregnancy detection by performing EIA of fecal sample extracts needs to be further investigated in a larger sample size of lions and tigers.

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Conflict of interest

The authors declare no conflict of interest.

Informed consent

This study was conducted with due permission of the Principal Chief Conservator of Forests (Wildlife) and Chief Wildlife Warden, Bengaluru vide No. PCCF(WL)/E2/MISC/CR-05/2020-2021 dated 07-03-2022.

References

1. Brown JL. Comparative endocrinology of domestic and nondomestic felids. Theriogenology 2006; 66 (1): 2536. https://doi.org/10.1016/j.theriogenology.2006.03.011
2. Andrews CJ, Thomas DG, Yapura J, Potter MA. Reproductive biology of the 38 extant felid species: a review. Mammal Review 2018; 49 (1): 1630. https://doi.org/10.1111/mam.12145
3. Bill LL, Jay FK. Monitoring ovarian function in captive and free-ranging wildlife by means of urinary and fecal steroids. Journal of Zoo and Wildlife Medicine 1991; 22 (1): 2331.
4. Bergfelt DR, Peter AT, Beg MA. Relaxin: a hormonal aid to diagnose pregnancy status in wild mammalian species. Theriogenology 2014; 82 (9): 11871198. https://doi.org/10.1016/j.theriogenology.2014.07.030
5. Schramm RD, Briggs MB, Reeves J. Spontaneous and induced ovulation in the lion (Pathera leo). Zoo Biology 1994; 13: 301-307. https://doi.org/10.1002/zoo.1430130403
6. Finkenwirth C, Jewgenow K, Meyer HH, Vargas A, Dehnhard M. PGFM (13,14-dihydro-15-keto-PGF2α) in pregnant and pseudo-pregnant Iberian lynx: a new non-invasive pregnancy marker for felid species. Theriogenology 2010; 73: 530540. https://doi.org/10.1016/j.theriogenology.2009.10.008
7. Dehnhard M, Naidenko SV, Jewgenow K. Comparative metabolism of PGFM (13,14-dihydro-15-keto-PGF2α) in feces of felids. Theriogenology 2014; 81: 733743. https://doi.org/10.1016/j.theriogenology.2013.12.007
8. Schwarzenberger F, Brown JL. Hormone monitoring: An important tool for the breeding management of wildlife species. Veterinary Medicine Austria 2013; 100: 209225.
9. Dehnhard M, Kumar V, Chandrasekhar M, Jewgenow K, Umapathy G. Non-Invasive Pregnancy Diagnosis in Big Cats using the PGFM (13,14-dihydro-15-ketoPGF2α) Assay. PLoS ONE 2015; 10 (12): e0143958. https://doi.org/10.1371/journal.pone.0143958
10. Mishra DP, Meyer HH, Prakash BS. Validation of a sensitive enzyme immunoassay for 13,14-dihydro-15-keto-PGF2α in buffalo plasma and its application for reproductive health status monitoring. Animal Reproduction Science 2003; 78: 33-46. https://doi.org/10.1016/S0378-4320(03)00047-2
11. Satoh K, Kawai Y, Mitsuhashi N, Kinoshita K, Sakamoto S. 1980. Prostaglandin F2 alpha metabolites in plasma and urine during pseudopregnancy in the rabbit. American Journal of Obstetrics and Gynecology 1980; 138: 748754. https://doi.org/10.1016/S0002-9378(16)32730-2
12. Tsutsui T, Stabenfeldt GH. Biology of ovarian cycles, pregnancy and pseudopregnancy in the domestic cat. Journal of Reproduction and Fertility 1993; 47: 2935.
13. Dehnhard M, Finkenwirth C, Crosier A, Penfold L, Ringleb J et al. Using PGFM (13,14-dihydro-15-keto-prostaglandin F2alpha) as a non-invasive pregnancy marker for felids. Theriogenology 2012; 77: 10881099. https://doi.org/10.1016/j.theriogenology.2011.10.011
14. Piper PJ, Vane JR, Wyllie JH. Inactivation of prostaglandins by the lungs. Nature 1970; 225 (5233): 600604. https://doi.org/10.1038/225600a0
15. Siemieniuch MJ, Jursza E, Szóstek AZ, Zschockelt L, Boos A et al. Placental origin of prostaglandin F2α in the domestic cat. Mediators of Inflammation 2014; 18. https://doi.org/10.1155/2014/364787
16. Kustritz MVR. Clinical management of pregnancy in cats. Theriogenology 2006; 66 (1): 145150. https://doi.org/10.1016/j.theriogenology.2006.03.018
17. Jewgenow K, Amelkina O, Painer J, Göritz F, Dehnhard M. Life cycle of feline corpora lutea: histological and intraluteal hormone analysis. Reproduction of Domestic Animals 2012; 47: 2529. https://doi.org/10.1111/rda.12033
18. Shille VM, Stabenfeldt GH. Luteal function in the domestic cat during pseudopregnancy and after treatment with Prostaglandin F2α. Biology of Reproduction 1979; 21 (5): 1217-1223. https://doi.org/10.1095/biolreprod21.5.1217
19. Little SE. Female reproduction. In: Little SE (editor). The Cat. 1st ed. Amsterdam, NL, Europe: Elsevier; 2012. pp. 11951227.
20. Graham LH, Goodrowe KL, Raeside JI, Liptrap RM. Non-invasive monitoring of ovarian function in several felid species by measurement of fecal estradiol-17β and progestins. Zoo Biology 1995; 14 (3): 223237. https://doi.org/10.1002/zoo.1430140304