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Non-invasive cortisol profile: A tool for reproductive stress in female tigers

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Abstract
Stress can be defined as any internal or external factor that affects the homeostasis of the animal. The factors by which it is caused are called as stressors. Stress is one of the known factors to have an impact on the reproductive physiology of the animals in terms of reduced fertility and sexual behaviour, as, it is believed that the stress hormones have inhibiting effect on gonadotropin releasing hormone. The wild felids are thought to get easily stressed while capturing, which, can have effect on the normal hormonal profile. Non- invasive collection of samples includes the collection of faeces and urine from the animals' enclosure or night cell in captivity without disturbing the normal routine of the animal. Faecal and urine samples of nine adult healthy animals which was deposited in the morning or 24 hours were collected on weekly intervals for 14 weeks from the night cell. Faecal samples were dried and extracted for cortisol by ethanol, whereas, urine puddles were collected from the floor by aspirating with the sterilized syringe. the least average value for faecal and urine cortisol has been found in the third week (4269 ± 932 pg/g) and tenth week (5541 ± 763 pg/mL), whereas, highest mean value for faecal and urine cortisol was observed on the second week (8652 ± 2484 pg/g) and fifth week (42077 ± 31906 pg/mL) respectively. It has shown a significant change between the groups on the first week of the trial for faecal cortisol, in which the lowest average value was exhibited by the G III (2444 ± 1309 pg/g) and the highest in G II (10310 ± 2542 pg/g). However, urine cortisol has shown a significant change between the groups on the fourth week of the trial with the highest value being exhibited by the G III (25250 ± 1380 pg/mL) and lowest in the G I (5183 ± 691 pg/mL). It was observed that the concentration of cortisol remains elevated in unmated group of animals as compared to mated animals.

Keywords: Stress, female tigers, reproduction, non-invasive, cortisol

Introduction
Stress is one of the known factors to have an impact on the reproductive physiology of the animals in terms of reduced fertility and sexual behaviour, as, it is believed that the stress hormones have inhibiting effect on gonadotropin releasing hormone. The wild felids are thought to get easily stressed while capturing, which, can have effect on the normal hormonal profile. Cortisol is considered to be the stress hormone that secreted in response to stress and can be used to diagnose the animal under stress. Therefore, the researchers have opted for the collection of samples non-invasively from the animal without disturbing their normal routine. Non- invasive collection of samples includes the collection of faeces and urine from the animals' enclosure or night cell in captivity without disturbing the normal routine of the animal. Narayan *et al.* (2013) [1] used faecal cortisol monitoring to investigate the stress physiology of two tiger sub-species, the Bengal and Sumatran tigers, held in two Australian zoos. This study demonstrated that tiger stress levels were significantly different between sexes and zoos, matching with previous studies that also found higher average stress levels in females than males for other felids. Parnell *et al.* (2014) [2] concluded in their article that faecal cortisol metabolites act as a prevailing marker for the physiological effects of the captive environment on the health and safety of big cats. Young *et al.* (2004) [3] described adrenocortical activity in carnivores including cheetah by non-invasive monitoring of glucocorticoid and have reported the overall mean, mean baseline and mean peak in cheetah as 751.1 ± 66.8, 618.5 ± 49.5 and 1850.3 ± 202.6 ng/g faeces respectively. Kinoshita *et al.* (2011) [4] jotted that the faecal cortisol metabolite concentration of three (A, B and C) female leopards were ranged from 0.01 to 0.99, 0.02 to 1.15 and 0.05 to 10.77 mg/g respectively.

The female C was found to have higher faecal cortisol metabolite throughout the study and have got failed to conceive on all four occasions of breeding season. Naidenko *et al*. (2019) [5] compared the extraction protocol for glucocorticoid with wet methanol (WM), dry methanol (DM) as well as dry ethanol (DE) and have found highest concentration by using WM followed by DM and DE. They estimated faecal glucocorticoid by using DE method of extraction in India and have shown that the highest level was found in Kanha Tiger Reserves (716 ± 184 ng/g) but it was not differed significantly from tigers' of Bandhavgarh and Sariska Tiger Reserves (383 ± 37 and 578 ± 127 ng/g respectively). Therefore, the present investigation was designed to know the cortisol concentration in relation to the various reproductive events prevailing in captive female tigers.

Materials and Methods

The research was carried out at Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (M.P.). Samples were collected from Maharaja Martand Singh Judeo White Tiger Safari and Zoo, Mukundpur, Satna (M.P.), Van Vihar National Park, Bhopal (M.P.), Kamla Nehru Prani Sangrahalaya, Indore Zoo, Indore (M.P.) and Gandhi zoological Park, Gwalior Zoo, Gwalior (M.P.). The experiment was conducted on nine adult healthy animals consisted of three groups (G I, G II and G III). Group G I (control) consist of 03 non-bred animals of less than 9 years of age, G II (control) consisted of 03 non-bred animals of more than 9 years of age and G III was consisted of 03 animals after mating irrespective of their age. Another two groups of mated and unmated animals of similar age were formed to compare stress in sexually active and inactive animals. The duration of experiment was one year. Faecal and urine samples deposited in the morning or 24 hours were collected on weekly intervals for 14 weeks from the night cell of animals. Faecal samples were dried and extracted for cortisol by ethanol, whereas, urine puddles were collected from the floor by aspirating with the sterilized syringe. These samples were then transferred to the vaccine carrier box and brought to the laboratory and stored at -20^{0}C for further analysis of hormone by ELISA.

Statistical Analysis: The data obtained during experiment were analyzed by IBM SPSS-24 statistical software program using one way ANOVA and t test.

Results and Discussions

The mean faecal and urine cortisol (Table 01 and 02; Figure 01 and 02) of the three groups were compared for the experimental period, the least average value for faecal and urine cortisol has been found in the third week (4269 ± 932 pg/g) and tenth week (5541 ± 763 pg/mL), whereas, highest mean value for faecal and urine cortisol was observed on the second week (8652 ± 2484 pg/g) and fifth week (42077 ± 31906 pg/mL) respectively. It has shown a significant change between the groups on the first week of the trial for faecal cortisol, in which the lowest average value was exhibited by the G III (2444 ± 1309 pg/g) and the highest in G II (10310 ± 2542 pg/g). However, urine cortisol has shown a significant change between the groups on the fourth week of the trial with the highest value being exhibited by the G III (25250 ± 1380 pg/mL) and lowest in the G I (5183 ± 691 pg/mL). The mean baseline faecal cortisol reported by Young *et al*. (2004) [3] and Naidenko *et al*. (2019) [5] were 618.5 ± 49.5 ng/g and 716 ± 184 ng/g respectively, which was more as compared to our findings, that might be resulted due to the ACTH challenge performed by the Young *et al*. (2004) [3] and Naidenko *et al*. (2019) [5].

Table 1: Mean ± SE of faecal cortisol (pg/g) in female tigers at weekly intervals

Weeks	Groups			Average
	G I	G II	G III	
1	5085^{ab} ± 1285	10310^{a} ± 2542	2444^{b} ± 1309	6647 ± 1489
2	7955 ± 4006	6185 ±1452	16213 ± 12197	8652 ± 2484
3	4361 ± 1645	4980 ± 1624	2306 ± 74	4269 ± 932
4	4234 ± 1897	5242 ± 635	3411 ± 1285	4573 ± 652
5	4235 ± 1296	3931 ± 943	8789 ± 4840	5247 ± 1317
6	6228 ± 2411	12303 ± 2848	4594 ± 1233	8351 ± 1696
7	2510 ± 255	8632 ± 1955	5878 ± 2503	6211 ± 1297
8	5609 ± 2109	7792 ± 1808	4354 ± 1091	6259 ± 1058
9	6786 ± 1656	6165 ± 1132	3848 ± 661	5793 ± 765
10	5774 ± 1297	5395 ± 872	5549 ± 2534	5541 ± 763
11	4818 ± 1688	7369 ± 1374	4753 ± 1588	5865 ± 898
12	5101 ± 1319	7847 ± 1974	5864 ± 2360	6436 ± 1067
13	5785 ± 2083	7501 ± 1053	3656 ± 912	5967 ± 901
14	5433 ± 1733	8729 ± 357	5391 ± 554	6796 ± 741

Means bearing different superscripts differ significantly between the groups (p<0.05).

Table 2: Mean ± SE of urine cortisol (pg/mL) in female tigers at weekly intervals

Weeks	Groups			Average
	G I	G II	G III	
1	33040 ±15894	7138 ± 3192	25405 ± 1535	21418 ± 6853
2	16862 ± 10271	5082 ± 1526	13760 ± 1580	12610 ± 4399
3	12903 ± 8399	3604 ± 1248	15875 ± 10935	11095 ± 4454
4	5183 ^{b} ± 691	7779 ^{b} ± 115	25250 ^{a} ± 1380	12737 ± 4005
5	81817 ± 75692	8645 ± 2156	15900 ± 3720	42077 ± 31906
6	54733 ± 48568	12410 ± 4010	18195 ± 12687	29410 ± 15950
7	61560 ± 53704	4611 ± 3133	14632 ± 6932	25177 ± 15355

8	5609 ± 2109	7792 ± 1808	4354 ± 1091	6259 ± 1058
9	6786 ± 1656	6165 ± 1132	5793 ± 661	5793 ± 765
10	5774 ± 1297	5395 ± 872	5549 ± 2534	5541 ± 763
11	4818 ± 1688	7369 ± 1374	4753 ± 1588	5865 ± 898
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Means bearing different superscripts differ significantly between the groups (p<0.05).

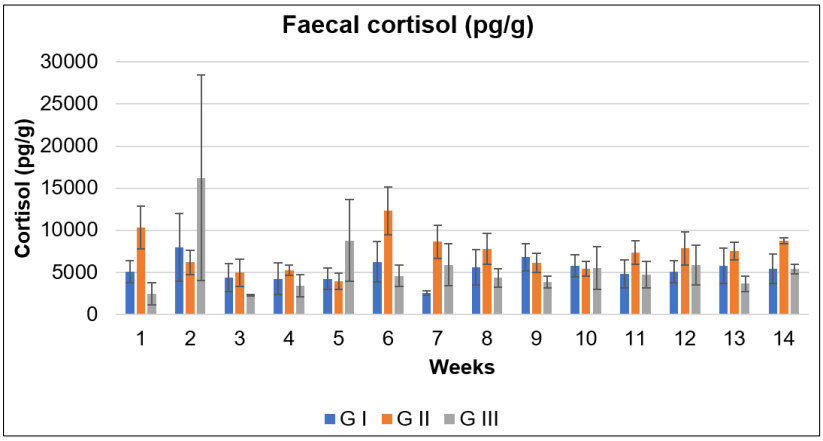

Fig 1: Mean ± SE of faecal cortisol (pg/g) in female tigers at weekly intervals

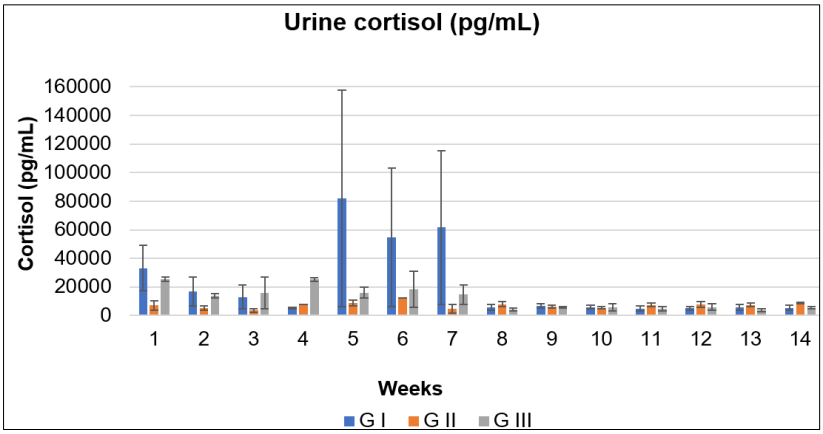

Fig 2: Mean ± SE of urine cortisol (pg/mL) in female tigers at weekly intervals

The faecal cortisol of the mated and unmated animals' groups (Table 03 and Figure 03) with the baseline and peak values in the respective groups were 1537 ± 769, 10808 ± 8876 pg/g and 2250 ± 900, 4876 ± 1282 pg/g. The urine cortisol of the mated and unmated animals' group (Table 04 and Figure 04) with the baseline and peak values in the respective groups were 2874 ± 1499, 31953 ± 22473 pg/mL and 4507 ± 1636, 64062 ± 56389 pg/mL.

Table 3: Mean ± SE and t value of faecal cortisol (pg/g) in mated and unmated female tigers at weekly intervals

Weeks	Groups		t value (p value)
	Mated animals	Unmated animals	
1	1629 ± 1111	3223 ± 1477	0.805 (0.458)
2	10808 ± 8876	3721 ± 1818	0.917 (0.401)
3	1537 ± 769	2817 ± 697	1.223 (0.276)
4	2274 ± 1357	2250 ± 900	0.015 (0.988)
5	8789 ± 4839	2704 ± 736	1.469 (0.202)
6	4594 ± 1233	3959 ± 1182	0.365 (0.730)
7	5878 ± 2502	3697 ± 1201	0.862 (0.428)
8	4354 ± 1091	2441 ± 832	1.424 (0.214)
9	3847 ± 661	4359 ± 926	0.417 (0.694)
10	5549 ± 2533	3292 ± 1101	0.907 (0.406)
11	4752 ± 1587	4523 ± 1403	0.108 (0.918)
12	5863 ± 2359	3266 ± 681	1.218 (0.278)
13	3655 ± 912	3916 ± 1001	0.185 (0.860)
14	5391 ± 554	4876 ± 1282	0.325 (0.759)

Table 4: Mean ± SE and t value of urine cortisol (pg/mL) in mated and unmated female tigers at weekly intervals

Weeks	Groups		t value (p-value)
	Mated animals	Unmated animals	
1	16936 ± 8514	25337 ± 13625	0.477 (0.654)
2	9173 ± 4676	13535 ± 7988	0.426 (0.688)
3	10583 ± 8237	10890 ± 6270	0.030 (0.977)
4	16833 ± 8454	4507 ± 1636	1.681 (0.154)
5	10600 ± 5718	64062 ± 56389	0.799 (0.460)
6	18194 ± 12686	30468 ± 24408	0.399 (0.706)
7	14632 ± 6931	31149 ± 28089	0.490 (0.645)
8	31953 ± 22473	44365 ± 41262	0.237 (0.822)
9	15426 ± 12217	11470 ± 6349	0.312 (0.768)
10	26123 ± 12481	12558 ± 10080	0.856 (0.431)
11	2874 ± 1499	37052 ± 20620	1.399 (0.221)
12	22421 ± 15256	26196 ± 14451	0.177 (0.867)
13	20996 ± 18002	23950 ± 12652	0.139 (0.895)
14	11783 ± 8627	9306 ± 6457	0.236 (0.823)

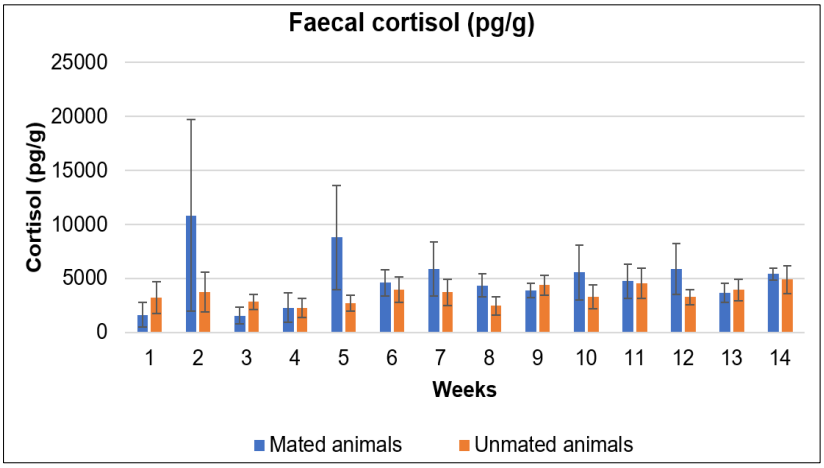

Fig 3: Mean ± SE of faecal cortisol (pg/g) in mated and unmated female tigers at weekly intervals

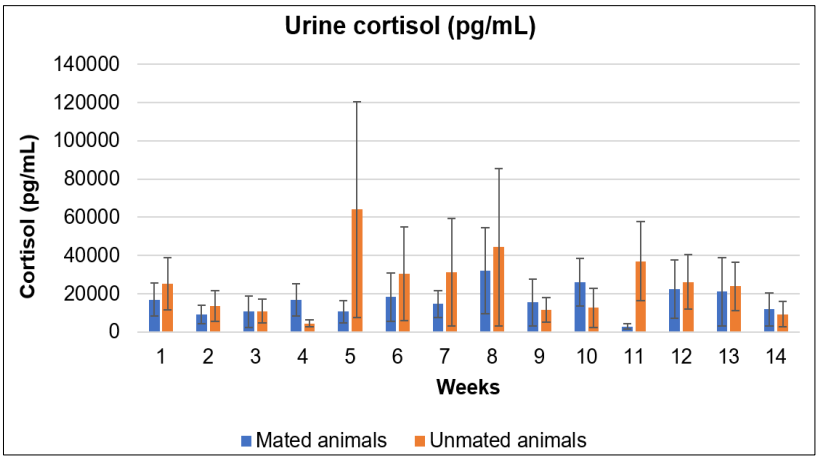

Fig 4: Mean ± SE of urine cortisol (pg/mL) in mated and unmated female tigers at weekly intervals

Conclusion

The remarkable concentration of cortisol was found in urine as compared to faecal samples of the female tigers. During the whole study period the cortisol was found to be high in concentration in urine samples of the sexually inactive animals (unmated). Cortisol correlated negatively with gonadotropins that impinge on the ovulatory and luteinization processes. The increase in cortisol can be seen in any acute and chronic stress. Hence, non-invasive cortisol profiling can be used as a tool for reproductive stress management in female tigers.

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