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Hormone Metabolites of E_2, Ig J Chain, LH and P_4 in Mated and Unmated Female Tigers (*Panthera tigris*)

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ABSTRACT

Background: Ovulation is a spontaneous process of releasing an egg from the ovaries of female animals on attainment of LH surge. However, in induced ovulators this could occur only after the stimulation by male animals or artificial induction. The big cats (*Panthera tigris*) are considered as induced ovulators in free range and semi- captivity.

Methods: Therefore, the present study was designed to observe the changes in the hormonal profile of the big cats before and following mating. A total of nine adult female tigers were grouped into two groups namely unmated and mated for the study from zoos and National parks of Madhya Pradesh. Faecal and urine samples of female tigers were taken on weekly basis for up to 14 weeks which is approximately 03 months of the study. The faecal and urine samples were processed and examined for the hormones *viz*. estrogen, immunoglobin (Ig) J chain, LH and progesterone were analysed by using ELISA plate reader. Urine constituents were analysed *viz*. urobilinogen, bilirubin, ketone, nitrite, glucose, blood, protein, leucocytes, pH and specific gravity in the urine sample by using urine analyser.

Result: Elevation in the concentration of the hormones have been observed in terms of estrogen, Ig J chain and LH, whereas, increase is observed in unmated group for up to 6th week pertaining to progesterone as well as very slight increase in the mated group after 6th week. Urinalysis revealed negative tests for urobilinogen, bilirubin, ketone, nitrite and glucose in all the samples. The samples were tested positive for presence of blood, protein and leucocytes in the urine sample, changes in the pH and slight in specific gravity have been found during the investigation.

Key words: Faeces, Urine, Hormonal profile, Mated, *Panthera tigris*, Unmated.

INTRODUCTION

Panthera tigris is being the world's largest cat in the world and are one of the critically in danger of extinction species. Female tigers are considered as an induced ovulators and certain studies also reported that their ovulation is induced type instead of spontaneous. Induced ovulation is a type of ovulation that requires a stimulus for the release of ovum. As a result of mating, neuroendocrine reflex leads to ovulation in induced ovulators. Penial intromission causes the distension of the posterior vagina that ultimately induces the release of gonadotropin releasing hormone (GnRH) from the hypothalamus. GnRH then acts on the anterior pituitary for the release of the Luteinizing hormone (LH), the surge of LH leads to the release of the ova or ovulation. Here, the LH surge takes place as a result of multiple matings in tigers with a frequency of around 200 mating normally required and that too for a longer period of time.

According to Kutzler (2007), he described in his study that felines are induced ovulators though 60 and 43 per cent of domestic cats and clouded leopards, respectively, that are unpaired, unmated and unstimulated female ovulate without any stimulation, also, seen in female leopards and lions that are housed together. It is suggestive of female-to-female interaction may induce ovulation. This natural induction of ovulation in this species have also shown the effect of social housing conditions. The housing categories that were categorized by Cabot *et al*. (2020) for their study and the presence of active corpora lutea reported in female tiger housed alone (66%), female housed with other females (85%) and female housed with at least one

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male (58%). Hormones play a pivotal role in the reproductive cycle management, the hormones of interest in induced ovulators are estrogen, progesterone and LH. The faecal estradiol concentration peaked in females housed with male for breeding as compared to females housed alone (Graham *et al*., 2006). Reproduction is a key to survival and hormones interaction results the processes. The basic reproductive endocrinology of felines is important to understand for the conservation and management of marvelous species. Therefore, the present study designed to observe the changes in hormonal profile of female tigers non-invasively before and after mating, so that the variation or difference in the hormone secreted can be depicted in this species that are considered as induced ovulators by means of faecal and urinary hormone metabolites as well as the urine physiological or pathological constituents with the following objectives:

1. Non-invasive reproductive hormone metabolites analysis in mated and unmated female tigers.
2. Changes in urine constituents of mated and unmated female tigers.

MATERIALS AND METHODS

The study was carried out at Department of Veterinary Physiology and Biochemistry, C.V.Sc. and A.H., N.D.V.S.U., Jabalpur (M.P.). Samples were collected from MMSJ White Tiger Safari and Zoo, Mukundpur, Satna (M.P.), Van Vihar National Park, Bhopal (M.P.), Kamla Nehru Prani Sangrahalya, Indore Zoo, Indore (M.P.) and Gandhi Zoological Park, Gwalior Zoo, Gwalior (M.P.).

Experimental design

Total of nine female tigers were considered and divided into two groups *viz*. mated (04) and unmated (05) groups for the experiment. The period of trial was one year.

Procedure

Sample collection and extraction

Faecal and urine samples of morning between 8:00 and 9:00 am or freshly voided samples were procured non-invasively from the night cell/ enclosure of the animals for up to 14 weeks. Samples (20 g) collected in zip lock bag were immediately taken to the vaccine carrier box to brought to the laboratory. Faecal samples were processed to be aliquot after drying at controlled temperature and processing involves ethanol extraction, they were stored at -20°C deep freezer. Hormones or hormone metabolites were estimated in faecal and urine samples by using standard ELISA kits. Urine or wet patch were aspirated from the floor and immediately kept in the vaccine storage box are then frozen in aliquots at -20°C temperature till further study. The extracted samples were then analyzed for estimation of hormones (Estrogen, Immunoglobulin J chain, LH and Progesterone) by enzyme linked immunosorbent assay (ELISA). Extraction of the faecal sample involves drying at controlled temperature, after drying ethanol extraction was carried out as per Dehnhard *et al*. (2012) for Estrogen and Progesterone. The extraction of the samples for the estimation of Ig J chain and LH was done with PBS in faeces collected as such from the premises, so that the protein content would not be hampered. They were vortexed for 30 minutes, then the supernatant was collected and aliquoted for further analysis.

Statistical analysis

The data obtained during experiment were analyzed by IBM SPSS-24 statistical software program using t test in both the groups (The normality was checked for t test) and Pearson correlation was done for the urinary constituents.

RESULTS AND DISCUSSION

Faecal and urine estrogen, immunoglobin J chain, luteinizing hormone and progesterone metabolites were analysed in the female tigers before (unmated) and after (mated) mating. The concentration of respective hormones and changes in urine physiological or pathological constituents of mated and unmated female tigers is presented in the succeeding tables.

Faecal and urine estrogen

The faecal estrogen of both the groups (Table 1) with the baseline and peak values 78±39 and 232±10 pmol/g in mated group, whereas, 53±31 and 161±18 pmol/g in the unmated group. Sixth, tenth and thirteenth week have shown a significant difference with slight decrease in the concentration sixth week onwards. The urinary estrogen metabolites of both the groups (Table 2) with the baseline and peak values 55±55 and 187±17 pmol/mL in mated group, whereas, 134±45 and 221±7 pmol/L in the unmated group of female tigers. Urinary estrogen in mated and unmated animals' group did show significant difference

Table 1: Mean (±SE) and t value of faecal estrogen (pmol/g) in female tigers on weekly basis in both the groups.

Weeks	Groups		t value
	Mated animals	Unmated animals	(p value)
1	124±63	150±18	0.444 (0.675)
2	124±63	155±5	0.561 (0.599)
3	112±58	103±37	0.149 (0.887)
4	100±52	161±18	1.235 (0.272)
5	78±39	160±25	1.877 (0.119)
6	232±10	58±33	4.293** (0.01)
7	212±37	71±41	2.425 (0.060)
8	182±12	71±41	2.251 (0.074)
9	197±42	86±49	1.617 (0.167)
10	215±22	55±32	3.755** (0.013)
11	174±42	76±25	2.083 (0.092)
12	159±27	70±44	1.557 (0.180)
13	174±20	53±31	2.914* (0.033)
14	136±45	128±20	0.195 (0.853)

t value with distinct superscripts within a row differ significantly between the groups (*p<0.05; **p<0.01).

on weeks sixth, tenth and thirteenth with p<0.001, p<0.01 and p<0.05, respectively as seen in faecal estrogen.

The slight decrease in the faecal estrogen is possibly due to increase in the progesterone concentration in unmated group. However, there was gradual rise in faecal estrogen concentration of mated group after mating was observed. Estrogen in higher concentration in estrus might be due to the formation of multiple follicles. Post estrus, the dominance is replaced by progesterone as a consequence of luteal function, this finding is in conjunction with Panda *et al.* (2017).

Being an induced ovulators, the species must require the presence of male counterpart for the successful surge of hormone and eventual ovulation. Some studies have reported that the visual, auditory and olfactory contact with the conspecific male is sufficient for the female to display a regular estrous cycle. The concentration of estrogen is influenced by the luteal secretion of progesterone. Estrogen remained elevated throughout the pregnancy in female tigers and this particular finding is in conjunction with the findings of Dehnhard *et al.* (2008).

Table 2: Mean (±SE) and t value of urine estrogen (pmol/L) in female tigers on weekly basis in both the groups.

Weeks	Groups		t value
	Mated animals	Unmated animals	(p value)
1	121±60	221±7	1.931 (0.111)
2	135±70	190±12	0.895 (0.412)
3	144±72	140±47	0.048 (0.964)
4	117±63	185±17	1.184 (0.289)
5	140±71	199±15	0.936 (0.392)
6	177±36	142±47	0.547 (0.608)
7	150±11	150±50	0.013 (0.990)
8	187±17	183±10	0.197 (0.852)
9	147±43	134±45	0.192 (0.855)
10	127±64	148±56	0.242 (0.819)
11	152±77	142±49	0.117 (0.911)
12	60±60	154±51	1.185 (0.289)
13	105±52	162±54	0.736 (0.495)
14	55±55	154±52	1.287 (0.254)

Table 3: Mean (±SE) and t value of faecal Ig J chain (µg/g) in female tigers on weekly basis in both the groups.

Weeks	Groups		t value
	Mated animals	Unmated animals	(p value)
1	3.2±1.7	7.8±1.1	2.239 (0.075)
2	8.7±5.8	14.0±5.7	0.622 (0.561)
3	6.4±3.2	12.8±1.8	1.878 (0.119)
4	7.0±3.6	12.8±3.3	1.152 (0.301)
5	10.3±2.9	10.7±2.7	0.101 (0.924)
6	14.3±6.5	9.3±2.8	0.780 (0.471)
7	10.9±4.1	11.9±2.5	0.217 (0.837)
8	8.7±2.7	12.1±3.0	0.809 (0.455)
9	5.6±1.0	9.9±0.9	2.997* (0.030)
10	9.3±2.4	9.0±1.6	0.101 (0.924)
11	11.5±2.0	7.8±1.4	1.575 (0.176)
12	7.4±1.4	11.7±2.3	1.437 (0.210)
13	7.9±0.9	12.6±3.5	1.089 (0.326)
14	8.1±0.3	13.7±3.5	1.300 (0.250)

t value with distinct superscripts within a row differ significantly between the groups (*p<0.05).

Faecal and urine immunoglobulin J chain (Ig J chain)

The faecal Ig J chain of both the groups (Table 3) did show significant difference on comparison of mated and unmated with the baseline and peak values 3.2±1.7 and 14.3±6.5 µg/g, whereas, 7.8±1.4 and 14.0±5.7 µg/g in the respective groups, significant difference on nineth week of the study has been found which might be due to maternal immunity.

The urine Ig J chain of the mated and unmated groups (Table 4) with the baseline and peak values 3.7±1.8 and 22.8±9.3 µg/mL, whereas, 9.3±4.0 and 24.5±5.5 µg/mL in the respective groups. It has shown significant difference on week nineth of the experimental period with p<0.05.

Immunoglobulin J chain abundance increased in gravid females compared to nonpregnant/nongravid females at 4 and 9 weeks after mating (natural breeding) that indicate the probable variation of maternal immunity in response to penetrating proceedings such as implantation and the increased secretory activity of the placenta.

Faecal and urine luteinizing hormone (LH)

The faecal LH of both the groups (Table 5) on comparison found no significant difference, the baseline and peak values were 2.0±1.0 and 9.1±3.6 ng/g, whereas, it was 2.8 ±1.9 and 11.8±8.3 ng/g in the mated and unmated groups, respectively.

The urinary LH of both the groups (Table 6) did not show any significant difference on comparison, the baseline and peak values were 1.8±0.9 and 31.1±21.3 ng/L in mated group, whereas, 6.1±3.4 and 25.9±18.7 ng/L in the unmated group.

Faecal and urine progesterone

The faecal progesterone metabolite (Table 7) with the baseline and peak values in mated group were 5947± 4208 and 25756±4871 pg/g, whereas, 14824±4569 and 29939±10960 pg/g, in unmated group.

The urine progesterone of both the groups (Table 8) with the baseline and peak values in both the groups were 1377±720, 7517±2831 pg/mL and 1195±147, 3103±1205 pg/mL, respectively. The significant change has been observed on comparison of groups at sixth, twelfth and thirteenth week of the study. The t value for urine progesterone in mated and unmated animals' group have shown a significant difference on week sixth, twelfth and thirteenth with p<0.05, p<0.01 and p<0.001 respectively.

The significant increase in the urinary progesterone observed at the particular weeks was might be due to persistence of corpus luteum (CL). The two-fold increase was showed at sixth week, whereas, the four times increase in the urinary progesterone concentration has been observed in the 12th and the 13th week. That might be due

to a larger number of CL resulted from several mating in due course multiple ovulations and might get retained in conditions like pseudopregnancy or actual pregnancy. Jesse *et al*. (2024) reported no significant change between the groups for faecal progesterone, whereas, urine progesterone has shown a significant change between the groups on the nineth, twelfth and thirteenth week of the trial with the highest value being exhibited by the G III on nineth week (7517±2831 pg/mL) and lowest in the G I at the same week (1163±586 pg/mL).

Urinalysis

Urinalysis of the found samples were also recorded to know the normal physiology in the female tigers. Analysis suggested certain some unusual changes in the urine parameters of the animal, the author thought that this unusual finding should be reported. The urine was physically examined for the colour and sniff test was performed after collection of the samples prior to urinalysis by using urine test strips in uriplus 200 urinalyzer. The test for urobilinogen, bilirubin, ketone, nitrite and glucose was found negative in all the samples. The samples were tested positive for presence of blood, protein and leucocytes in the urine sample, changes in the pH and slight in specific gravity have been found during the investigation. Hence, the mean of the week of all the groups and correlation of the urine constituents is being presented in the succeeding tables and figures under subheading.

Table 4: Mean (±SE) and t value of urine Ig J chain (µg/mL) in female tigers on weekly basis in both the groups.

Weeks	Groups		t value
	Mated animals	Unmated animals	(p value)
1	3.7±1.8	11.3±4.6	1.316 (0.245)
2	12.1±6.0	18.8±8.1	0.609 (0.569)
3	15.9±8.3	13.1±5.2	0.296 (0.779)
4	15.2±12.7	17.1±7.8	0.134 (0.898)
5	7.5±4.1	24.5±5.5	2.306 (0.069)
6	13.5±2.9	11.3±4.2	0.395 (0.709)
7	11.0±2.2	13.6±5.4	0.381 (0.719)
8	15.2±6.7	17.1±1.8	0.307 (0.771)
9	14.9±3.3	10.6±3.7	0.843 (0.437)
10	14.8±1.7	9.3±4.0	1.082 (0.329)
11	14.8±2.3	13.4±1.1	0.561 (0.599)
12	22.8±9.3	11.8±4.4	1.178 (0.292)
13	17.5±5.4	10.7±3.7	1.079 (0.330)
14	15.8±5.3	13.9±4.6	0.265 (0.802)

Table 5: Mean (±SE) and t value of faecal LH (ng/g) in female tigers on weekly basis in both the groups.

Weeks	Groups		t value
	Mated animals	Unmated animals	(p value)
1	2.0±1.0	5.9±3.0	1.035 (0.348)
2	3.5±3.0	5.6±4.3	0.364 (0.730)
3	3.0±1.5	5.3±2.6	0.695 (0.518)
4	5.6±2.8	3.8±1.8	0.544 (0.610)
5	7.3±3.9	9.2±8.7	0.178 (0.866)
6	3.8±0.6	5.2±2.6	0.451 (0.671)
7	3.6±1.0	11.8±8.3	0.824 (0.447)
8	5.4±3.0	4.0±1.5	0.457 (0.667)
9	8.7±4.4	5.1±1.3	0.900 (0.409)
10	3.3±1.4	5.9±2.3	0.874 (0.422)
11	9.1±3.6	5.0±2.1	1.021 (0.354)
12	2.1±0.9	9.0±4.1	1.400 (0.220)
13	6.7±0.7	4.1±2.7	0.763 (0.480)
14	4.2±0.4	2.8±1.9	0.571 (0.593)

The pulsatile secretion of LH might be missed to track in the urine samples however the weekly samples have given the slight increased concentration indicative of estrus and mating.

Urinary blood

The mean of blood in urine of mated and unmated groups for the trial period has been shown in the Table

Table 6: Mean (±SE) and t value of urine LH (ng/L) in female tigers on weekly basis in both the groups.

Weeks	Groups		t value
	Mated animals	Unmated animals	(p value)
1	4.8±2.7	7.8±4.0	0.562 (0.598)
2	6.2±3.5	19.1±16.7	0.641 (0.550)
3	1.8±0.9	8.6±4.0	1.426 (0.213)
4	6.4±5.9	7.6±2.6	0.207 (0.844)
5	7.3±6.0	12.0±10.1	0.362 (0.732)
6	6.6±1.1	6.1±3.4	0.129 (0.902)
7	7.8±0.7	14.5±10.1	0.548 (0.607)
8	8.0±2.8	6.8±2.4	0.291 (0.783)
9	7.5±3.6	4.2±1.5	0.905 (0.407)
10	3.9±0.8	7.0±3.9	0.653 (0.543)
11	31.1±21.3	7.7±2.8	1.288 (0.254)
12	5.6±0.6	25.9±18.7	0.913 (0.403)
13	9.9±1.5	6.9±3.2	0.721 (0.503)
14	7.3±0.15	7.3±2.3	0.013 (0.990)

Table 7: Mean (±SE) and t value of faecal progesterone (pg/g) in female tigers on weekly basis in both the groups.

Weeks	Groups		t value
	Mated animals	Unmated animals	(p value)
1	9293±5611	20255± 3645	1.719 (0.146)
2	10275±6409	22619±8393	1.094 (0.324)
3	10480±5348	26158±13283	0.959 (0.381)
4	5947±4208	19072±5352	1.811 (0.130)
5	15841±5294	19750±4209	0.586 (0.583)
6	15216±1357	14824±4569	0.071 (0.946)
7	17576±4184	15501±2892	0.424 (0.689)
8	15234±2615	15157±3358	0.017 (0.987)
9	15222±3546	27430±6620	1.457 (0.205)
10	18060±5893	19264±3594	0.185 (0.861)
11	25756±4871	15973±2324	1.990 (0.103)
12	17877±4654	24342±9822	0.527 (0.620)
13	15965±3090	27371±15023	0.635 (0.553)
14	22927±4488	29939±10960	0.519 (0.626)

9. The overall mean of both the groups was compared for the experimental period; it was found to be 25.68±10.53 and 70.61±14.85 erythrocytes/µL in mated and unmated female tigers respectively.

Blood is generally considered as a pathological constituent of urine, which is usually not present in the urine of individual animal, if it is, then there might be some infection present in the urinary tract. But, in case of felines, big cats precisely, the presence of blood has been seen in count. There was no significant change have been found in all the classified groups, statistically. However, we do find a least number of erythrocytes normally present in the urine of female tigers are 5.5±2.7 erythrocytes/µL. The normal value of presence of blood in urine is not documented to the best of our knowledge, hence, 3 to 8 erythrocytes/µL should be noted to present normally in the urine of tigers, count can be as high as 92.7±34.0 erythrocytes/µL normally. Similar to these findings, Chew (2012) and Yadav *et al*. (2020) mentioned in their review article on feral cat.

Table 8: Mean (±SE) and t value of urine progesterone (pg/mL) in female tigers on weekly basis in both the groups.

Weeks	Groups		t value
	Mated animals	Unmated animals	(p value)
1	1377±720	1195±147	0.291 (0.783)
2	2927±1832	1626±862	0.706 (0.512)
3	3404±2081	1394±451	1.104 (0.320)
4	5169±3110	2751±1302	0.799 (0.460)
5	5094±2566	2081±1114	1.196 (0.285)
6	7351±907	2712±1352	2.618* (0.047)
7	6596±4210	1742±794	1.331 (0.241)
8	6619±3337	1708±456	1.726 (0.145)
9	7517±2831	1489±527	2.461 (0.057)
10	5503±2943	2864±1461	0.877 (0.421)
11	4850±2093	3103±1205	0.774 (0.474)
12	7230±1712	1652±575	3.516** (0.017)
13	7139±1352	1629±293	4.654** (0.01)
14	5660±2262	2330±619	1.640 (0.162)

t value with distinct superscripts within a row differ significantly between the groups (*p<0.05; **p<0.01).

Table 9: Mean (±SE) of urine constituents in female tigers at weekly intervals for fourteen weeks.

Urine Constituents	Groups	
	Mated animals (n = 03) (ns = 42)	Unmated animals (n = 04) (ns = 56)
Blood (erythrocytes/µL)	25.68±10.53	70.61±14.85
Protein (mg/dL)	154.13±22.21	151.41±15.92
Leucocytes (leucocytes/µL)	135.11±19.90	20.71±3.77
Specific gravity	1.012±0.00	1.014±0.00
pH	8.11±0.11	7.95±0.11

"n" is number of animals; "ns" is number of samples.

Urinary protein

The mean of protein in urine of mated and unmated groups for the experimental period has been shown in the Table 9. Unlike other species, protein is present in the feline urine, which is due to the presence of cauxin protein, might also have dietary influence. The normal concentration of which is not known though. However, as per our investigation the normal range could be in between 13 to 77 mg/dL, slight increase in the concentration of physiologic proteinuria, that can be as high as 150-250 mg/dL. This might be due to the stage of estrus which is characterized by the presence of pheromones in the urine of the animal with distinguished odour of urine, the animal might be having reproductive stress or strenuous exercise, that results in transient, increased permeability of glomeruli to plasma proteins. This finding is in conjugation with the reported findings of Rizzi (2014).

Urinary leucocytes

The mean of leucocytes in urine of mated and unmated groups for the experimental period has been shown in the Table 9. The number of leucocytes that was found during the investigation was 08 - 156 leucocytes/µL. Presence of leucocytes in urine, typically, neutrophils should be below 6 in numbers as the pyuria is due to contamination from prepuce or vagina, genital tract and urinary tract inflammation or infection (Rizzi, 2014). Yadav *et al*. (2020) mentioned in their review article, that the permissible count of white blood cells is 5, more than 5 may be accompanied with the infection of urinary tract. The higher count could be suggestive of genitourinary infection and /or coitus in case of bred animals.

Urinary pH

The mean of urinary pH of mated and unmated groups for the experimental period has been shown in the Table 9. The normal range of urinary pH mentioned by Yadav *et al*. (2020) in dogs and cats was 6-7.5. The range in our study was found to be between 7.63-8.11, the higher side of urinary pH could be due to the presence of blood. No literature on the urinary pH of the tigers has been found.

Table 10: Correlation coefficient of urine constituents during the experimental period.

Variable	Blood	Protein	Leucocytes	Specific gravity
Blood	-			
Protein	0.286	-		
Leucocytes	0.066	0.649	-	
Specific gravity	-0.454	-0.469	-0.797	-
pH	0.578	0.636	0.832*	-0.866*

*p<0.05 (2- tailed).

Urinary specific gravity

The mean of urinary specific gravity of mated and unmated groups for the trial period has been shown in the Table 9. The mean of the weeks of mated and unmated animals' group was 1.012±0.00 and 1.014±0.00. There is no literature on the urinary specific gravity in tigers have been found. However, Watson *et al*. (2015) have mentioned the urinary specific gravity in cats was in between 1.035-1.060, which is quite changed from our finding.

Correlation of urine constituents

The Pearson correlation between the urine constituents have been done to see the relationship between the variables and is being presented in the Table 10.

It was observed that the urine specific gravity is negatively correlated with blood, protein, leucocytes and pH; among these the significant change has been observed while correlating it with pH.

CONCLUSION

This study underscores the utility of non-invasive biomarkers such as estrogen, progesterone and immunoglobulin J chain in understanding the reproductive physiology of female tigers. In conclusion to the results of the study obtained, the changes have been observed pertaining to the hormonal concentration in both the groups of female tigers. Estrogen remained elevated after mating and also during the complete gestation period. Progesterone have shown two to four-fold increase in concentration in mated females. Slight increase in concentration of LH was observed; Ig J chain increased in mated females compared to unmated females. Also, the urinary constituents have shown very different scenario in these species, which the author has reported. Vast variation has been found in the pH, presence of blood and high protein concentration in urine of the tigers, which can be treated as pathological, but they are physiological or normal in these species. Hence, the reproductive physiology and urinalysis of tigers could be a tool to understand the different phases of estrous cycles.

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Conflict of interest

The experiment was conducted as per the guidelines of Institutional Animal Ethics Committee (IAEC) vide order no. 56/IAEC/Vety. /2020 dated 27/10/2021 and Principal Chief Conservator Forest (PCCF), M.P. approval no. Draft men – II/Research/2890 dated 30.03.2021. The authors declare that they have no conflict of interest in publishing this article.

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