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Can Non-invasive Hormone Profiling in Female Tigers (*Panthera tigris*) could Differentiate True Pregnancy and Pseudopregnancy

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ABSTRACT

Background: The tigers (*Panthera tigris*) being the world's largest cat are one of the critically endangered species which are at the verge of extinction. The pseudopregnancy or false pregnancy or pseudocyesis is a syndrome which is characterized by the presence of signs of pregnancy in a non-pregnant animal. False lactation or nervous lactation are the terms which are used to refer pseudopregnancy as there is very low quantities of milk production is seen. This condition can be differentiated with true pregnancy by using hormones and by correlation of behavioral symptoms of pregnancy with hormonal profiles for confirmation of pregnancy in female tigers.

Methods: Total seven animals were used in the experiment and was divided into three groups (G I, G II and G III). Group G I consist of 03 non-bred animals of less than 9 years of age, G II 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. Faecal samples were collected non-invasively from enclosures weekly up to 14 weeks. Only fresh samples (deposited within 24 h) was chosen, urine puddles were aspirated from the floor. The extracted samples were then analysed for estimation of relaxin, progesterone and prostaglandin F_2 alpha metabolite (PGFM) by enzyme linked immunosorbent assay (ELISA).

Result: Relaxin the hormone for early detection of pregnancy in felines gives elevated concentration from 49 days after mating and attains a peak during parturition, whereas, no elevation or peak was observed in pseudopregnant animals. Progesterone, in true pregnancy remains elevated from 7th week onwards after mating, it gets dropped after 7th week with wide fluctuation in pseudopregnant animals. PGFM elevation observed after nine weeks of gestation in true pregnancy whereas, no elevation will be observed in pseudopregnant tigress.

Key words: Faeces, Hormones, Pregnancy, Pseudopregnancy, Tigers, Urine.

INTRODUCTION

Certain critically endangered mammalian species depend on successful captive breeding programmes to thrive. To maintain the greatest possible biodiversity, breeding in captivity also necessitates the use of contemporary assisted reproductive techniques, yet understanding of reproductive physiology is frequently lacking in these species of animals. As a result, non-invasive hormone monitoring of the faeces and urine has grown in importance as a reproductive management technique.

The tigers (*Panthera tigris*) being the world's largest cat are one of the critically endangered species which are at the verge of extinction. There are currently six known subspecies of tigers, but three have been vanished in the past 80 years. The subspecies that are currently recognized are the South-China, Bengal, Indochinese, Sumatran, Amur and Indochinese subspecies. Reproductive or sexual activities of tigers, such as mating, can take place all year round, but in some parts of the world with temperate climate, their sexual activities seem to be most prevalent from November to April. They are induced ovulators and the induction of ovulation is carried out by the copulation. Sexual receptivity of a female is only of few days and during that time, mating is very often.

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The pseudopregnancy or false pregnancy or pseudocyesis is a syndrome which is characterized by the presence of signs of pregnancy in a non-pregnant animal.

False lactation or nervous lactation are the terms which are used to refer pseudopregnancy as there is very low quantities of milk production is seen. This condition can be differentiated with true pregnancy by using hormones. In this non-pregnant luteal phase, the faecal progestin elevated for a shorter duration of approximately one-half to two-thirds of the gestation length. Relaxin and progesterone are said to be hormones of pregnancy in felines, between the two, relaxin is considered as early diagnosis factor of pregnancy in felines. The diagnosis of pregnancy in tigers by common methods as in domestic animals is quite difficult and stressful to the wild animals. The non-invasive technique uses analyses of faecal and urinary steroid metabolites for long-term assessment of ovarian activity in females, in particular, for pregnancy diagnosis and parturition prediction in captive species. It is difficult to identify a pregnant female tiger because there might be none of the morphological signs have been displayed by the animal in the earlier phase of the pregnancy. Therefore, the present study was designed to know the reproductive status of female tigers non-invasively after mating that whether underwent true pregnancy or pseudopregnancy by means of faecal and urinary hormones.

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.) for fourteen weeks of experimental period from November 2021 to February 2022.

Experimental design

The experimental design was consisted of three groups *viz.* G I 03 non-bred animals of less than 9 years of age, G II 03 non-bred animals of more than 9 years of age and G III 03 animals after mating irrespective of their age. The duration of experiment was one year.

Sample collection and extraction

The urine and faecal samples of the tigress were collected in the morning between 8 and 9 am. Faecal samples were collected non-invasively from individual enclosures weekly for up to 14 weeks of pregnancy. Only fresh samples (deposited within 24 h) was chosen. Samples, about 20 g in size, were collected in individual plastic bags and immediately transferred in the vaccine storage box (to maintain the temperature for longer period of time of journey). These biological materials collected was brought to and processed at the laboratory for extraction and analysis of the samples', extracted samples were then stored at -20°C in a deep freezer. Urine puddles was aspirated from the floor and immediately kept in the vaccine storage box, then frozen in aliquots at -20°C temperature till further analysis. Extraction of the faecal sample involves drying at controlled temperature, after drying ethanol extraction was carried out as per Dehnhard *et al*. (2012). The extracted samples were then analysed for estimation of hormones (relaxin; progesterone and prostaglandin F_2 alpha metabolite, PGFM) by enzyme linked immunosorbent assay (Arbor Assays ELISA kit). For any changes in the behaviour of animal resulted due to any reproduction perception by the animal was monitored and recorded through CCTV (closed circuit television).

Statistical analysis

The data obtained during experiment were analyzed by IBM SPSS-24 statistical software program using one way ANOVA in three groups.

RESULTS AND DISCUSSION

True pregnancy and pseudopregnancy in big cats can be diagnosed on the basis of hormones and its metabolites in faecal and urine samples *viz.* relaxin, progesterone and PGFM. Since, a smaller number of references on the behaviour of pregnancy of tigers have been found, the references included here are on the basis of the experiences of the zoo keepers as well as from the unpublished articles. The concentration of the hormones obtained during experimental period is presented in the tables.

Faecal and urinary relaxin

The mean faecal and urine relaxin of the three groups in (Table 1 and 2) were compared for the experimental period, the least average value for faecal and urine relaxin has been found in the sixth week (870.6±125.5 pg/g) and thirteenth week (1307.7±56.4 pg/mL), whereas, highest mean value for faecal and urine relaxin was observed on the fourteenth week (1826.8±784.7 pg/g) and twelfth week (2987.7±1474.7 pg/mL), respectively. It has shown a significant change between the groups on the first, fifth, seventh, eighth, nineth, tenth, eleventh and twelfth week of the trial for faecal relaxin, in which the lowest average value was exhibited by the G I and the highest in G III. However, urine relaxin 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 (1676.7±133.2 pg/mL) and lowest in the G I (1183.2±90.1 pg/mL).

Corpus luteum is the primary source of relaxin in both pregnant and non-pregnant animals. Increased plasma level is seen during pregnancy, in which case, it is produced by the decidua and placenta its highest level attained prior to parturition. Its visible concentration observed on 4^{th} week after mating of animal with a decline on 5^{th} and again a rise and elevation observed from 7^{th} week onwards. However, the reported mean baseline faecal relaxin by Harris *et al*. (2008) were not in agreement to our findings.

Faecal and urinary progesterone

The mean faecal and urine progesterone of the three groups (Table 3 and 4) were compared for the experimental

period, the least average value for faecal and urine progesterone has been found in the fourth week (11845±2747 pg/g) and first week (1196±231 pg/mL), whereas, highest mean value for faecal and urine progesterone was observed on the fourteenth week (20239±4531 pg/g) and sixth week (4661±1143 pg/mL) respectively. No significant change has been observed 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 same week (1163±586 pg/mL).

The mean baseline faecal progesterone reported by Panda *et al*. (2017) were similar to our finding in the respective reproductive status of animal. Qaio *et al*. (2019) reported urine progesterone and their finding was more as compared to our findings. The significant increase in the urine progesterone observed at the respective weeks was might be due to persistence of corpus luteum (CL). The sixth week had showed a two-fold increase, whereas, the four-fold increase in the urine progesterone concentration has been observed in the twelfth and the thirteenth week. This might be due to a greater number of CL resulted from multiple mating in due course multiple ovulations and might get retained in conditions like pseudopregnancy or actual pregnancy.

Faecal and urinary PGFM

The mean faecal and urine PGFM of the three groups (Table 5 and 6) were compared for the experimental period, the

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

Weeks	Groups			Average
	G I	G II	G III	
1	837.4^{b}±93.9	1024.9^{ab}±52.5	1213.0^{a}±180.3	1009.4±67.4
2	936.3±45.2	997.9±166.6	1163.0±59.3	1018.6±72.4
3	905.1±108.5	1036.8±83.0	1128.7±341.2	1015.8±89.9
4	938.4±83.9	1008.9±126.9	1373.9±266.8	1076.7±95.2
5	860.0^{b}±33.4	996.2^{ab}±77.3	1291.2^{a}±242.6	1024.5±78.4
6	567.0±222.8	1063.3±123.5	954.3±317.5	870.6±125.5
7	813.8^{b}±95.7	1094.3^{ab}±67.8	1456.9^{a}±210.3	1091.5±94.4
8	724.7^{b}±163.1	1038.8^{b}±77.4	1438.0^{a}±123.8	1033.9±103.1
9	813.7^{b}±184.5	1065.5^{b}±68.6	1534.1^{a}±187.8	1098.7±110.5
10	942.5^{b}±109.1	1051.7^{ab}±41.3	1563.9^{a}±352.2	1143.4±111.9
11	883.3^{b}±83.2	1188.9^{b}±74.9	1631.0^{a}±204.5	1197.5±103.2
12	951.6^{b}±93.4	1216.6^{b}±69.9	2339.6^{a}±701.7	1409.0±226.6
13	887.8±167.8	1189.4±46.26	3758.7±2118.8	1731.2±577.1
14	711.8±155.3	1092.4±58.0	4537.4±2921.8	1826.8±784.7

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

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

Weeks	Groups			Average
	G I	G II	G III	
1	1277.3±75.48	1171.8±25.1	1603.6±377.1	1360.1±144.3
2	1304.7±124.9	1337.0±48.7	1281.3±139.4	1307.7±56.4
3	1579.6±33.2	1244.1±135.4	1358.5±93.4	1370.9±73.2
4	1183.2^{b}±90.1	1406.2^{ab}±100.0	1676.7^{a}±133.2	1422.0±89.8
5	1279.4±189.1	1413.4±167.4	1348.0±29.7	1355.4±69.0
6	1364.7±87.3	1412.7±142.0	1166.8±61.3	1308.4±67.8
7	1145.2±138.7	1403.9±159.6	1665.7±113.0	1437.4±103.3
8	1606.0± 45.7	1396.6±39.6	1547.5±154.0	1516.7±57.0
9	1437.7±264.7	1166.2±101.2	1659.9±254.9	1419.2±130.8
10	1243.6±42.5	1351.4±104.7	1309.9±151.8	1308.9±62.9
11	1556.1±131.8	1284.0±37.1	1349.7±4.3	1396.6±56.9
12	1430.6±96.96	1646.3±247.1	5367.2±3961.9	2987.7±1474.7
13	1561.3±117.6	1460.35±24.4	4691.1±3414.8	2697.1±1261.3
14	1155.5±62.7	1294.0±133.8	5809.6±4616	2952.7±1727.7

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

least average value for faecal and urine PGFM has been found in the fourth week (8960.1±1203.6 pg/g) and second week (7110.2±1967.5 pg/mL), whereas, highest mean value for faecal and urine PGFM was observed on the thirteenth week (24579.3±6295.8 pg/g) and thirteenth week (21766.8±13574.5 pg/mL) respectively. The significant change has been observed between the groups for faecal PGFM on second and fourth week. Urine PGFM did show significant change between the groups on first and nineth week of the investigation.

The present findings are in conjunction with the findings reported by Finkenwirth *et al*. (2010) and Dehnhard *et al*. (2012). The mean baseline faecal PGFM reported by Dehnhard *et al*. (2012) in Sumatran tigers differ in maximum levels of 0.4 to 2.0 µg/g faeces PGFM, whereas, the mean baseline urinary PGFM reported by Finkenwirth *et al*. (2010) was 1.5 ng/mL. Faecal as well as urinary PGFM concentration attain a significant rise from the baseline during the end of the pregnancy peaking towards parturition. Elevation of PGFM observed after nine weeks of gestation in big cats whereas, it is 2-4 days prior to parturition in female dogs. Unlike pregnant females, pseudopregnant and nonpregnant females does not show this elevated pattern. In addition, faecal and urine PGFM concentrations decrease drastically at the time of abortion and premature birth, indicating a strong relationship of this hormone with the maintenance of full-term pregnancy.

Behavioural symptoms of pregnancy

The decreased frequency of scent marking has been observed in the gravid animal. The animal has been noticed that; a particular place has been selected to rest

Table 3: Mean±SE of faecal progesterone (pg/g) in female tigers at weekly intervals.

Weeks	Groups			Average
	G I	G II	G III	
1	18816±4559	13193±2641	9293±5611	14092±2368
2	20550±9425	10843±2825	10275±6409	13936±3625
3	24307±14021	8452±1532	10480±5348	14244±4918
4	16663±6002	11529±3607	5947±4208	11845±2747
5	17677±5045	10718±2226	15841±5294	14319±2285
6	12786±4209	14756±4959	15216±1357	14214±2348
7	15413±2880	15551±5438	17576±4184	16011±2470
8	13281±2938	17107±6209	15234±2615	15364±2680
9	23849±7928	14133±3475	15222±3546	17644±3143
10	16820±4487	17311±4333	18060±5893	17335±2504
11	14088±3370	20240±8661	25756±4871	19568±3908
12	23342±10378	16284±5662	17877±4654	19035±4063
13	25675±15694	14542±4277	15965±3090	18609±5281
14	28625±11697	11917±3864	22927±4488	20239±4531

Table 4: Mean±SE of urine progesterone (pg/mL) in female tigers at weekly intervals.

Weeks	Groups			Average
	G I	G II	G III	
1	1308±133	902±206	1377±720	1196±231
2	1749±1207	1052±107	2927±1832	1909±690
3	1537±605	1401±599	3404±2081	2114±725
4	3329 ±1650	2808±2047	5169 ±3110	3768 ±1229
5	2562±1420	2452±1688	5094±2566	3369±1068
6	3296±1725	3336±2543	7351±907	4661±1143
7	2053±1034	2092±1102	6596±4210	3580±1495
8	1694±645	1791±513	6619±3337	3368±1282
9	1163^{b}±586	1682^{ab}±633	7517^{a}±2831	3454±1329
10	3250±1993	2711±1493	5503±2943	3821±1192
11	3623±1537	3439±2334	4850±2093	3971±1032
12	1563^{b}±804	2435^{b}±1176	7230^{a}±1712	3743±1090
13	1669^{b}±411	2024^{b}±857	7139^{a}±1352	3611±1004
14	2645±755	1738±795	5660±2262	3348±937

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

Table 5: Mean±SE of faecal PGFM (pg/g) in female tigers at weekly intervals.

Weeks	Groups			Average
	G I	G II	G III	
1	18836.7 ±5513.0	10276.0±3607.0	8755.3±3519.2	12749.4 ±2648.4
2	19245.5^{a}±5583.2	7559.0^{ab}±2196.7	3560.3^{b}±719.7	10454.8±2707.0
3	14818.2±4171.9	9004.8 ±2401.3	8922.6±1379.5	10922.0±1797.9
4	11670.2^{a}±2384.6	9188.4^{ab}±1125.3	4966.3^{b}±1833.2	8960.1±1203.6
5	11314.2±2629.3	11020.2±2074.8	8816.6±1846.5	10567.3±1236.9
6	9691.7±3957.0	19790.2±6163.5	10675.3±2724.3	14145.3±3098.1
7	9188.5±2124.6	13756.8±5326.2	11024.3±3164.6	11550.9±2350.2
8	13556.2±2585.7	18247.4±4768.1	24298.6±17298.8	18196.5±4376.5
9	13432.5±1547.7	20606.0±6502.3	26952.6±20464.6	19801.5±5298.7
10	20939.0±8531.6	14127.0±5165.4	32410.0±17320.9	20968.4±5389.3
11	20515.5±11055.2	16747.6±8329.4	30447.3±20306.7	21428.5±6562.8
12	17161.5±8739.2	20942.6±9367.2	30309.0±22166.4	22023.8±6700.2
13	24942.0±9349.1	18561.6±8107.3	34125.3±20056.9	24579.3±6295.8
14	23640.2±9480.2	22567.8±9206.8	28131.6±20732.2	24316.2±6404.0

Means bearing different superscripts (a, b) are showing significant difference between the groups ($p<0.05$).

Table 6: Mean±SE of urine PGFM (pg/mL) in female tigers at weekly intervals.

Weeks	Groups			Average
	G I	G II	G III	
1	2349.0^{b}±2027.0	4293.0^{b}±2098.9	15771.6^{a}±3890.4	8111.5±2711.2
2	4001.3±1901.0	4882.0±3849.4	12447.33±2543	7110.2±1967.5
3	4317.5±3987.5	12815.6±11214.2	30548.3±18593.9	17340.8±8220.9
4	2213.6±1842.7	10895.6±9218.9	13866.3±3837.4	8991.8±3412.2
5	2091.5±1752.5	29728.0±26331.8	10830.3±2795.6	15732.2±9677.9
6	866.5±471.5	20068.6±17509.2	18916.6±1367.7	14836.1±6510.3
7	1502.5±945.5	4102.3±2418.9	19048.0±10396.5	9057.0±4576.0
8	935.3±455.5	4623.3±2168.4	22527.3 ±12588.3	9362.0±4973.0
9	720.5^{b}±392.5	4424.0^{ab}±2610.5	22486.0^{a}±7442.8	10271.3±4444.4
10	1612.0±1026.0	3180.6±1227.1	15118.3±7403.3	7265.1±3377.9
11	946.6±364.5	4550.3±2099.9	26566.3±16698.1	10687.7±6296.2
12	1726.0±1280.0	11356.6±9359.6	42420.0±32948.8	20597.7±12983.3
13	1218.0±727.0	11333.0±9749.8	45900.0±33737.2	21766.8±13574.5
14	1942.0±1383.0	5434.6±3488.6	49406.0±35720.3	21050.7±14396.4

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

upon. Most of the time, the animal, was seen to be in recumbency and has spent time for sleeping. The enlargement of abdomen was seen in the last stage of pregnancy with protruding teats. The gradual restlessness of the animal was seen and has been recorded, the continuous avoidance of male by the female has been perceived. Later, it started search of secluded place for cubbing, so that, the cubs would be saved from any noxious deed.

CONCLUSION

Relaxin the hormone for early detection of pregnancy in felines gives elevated concentration from 49 days after mating and attains a peak during parturition, whereas, no elevation or peak is observed in pseudopregnant animals. Progesterone, in true pregnancy remains elevated from 7th week onwards after mating. It gets dropped after 7th week with wide fluctuation. PGFM elevation observed after nine weeks of gestation in true pregnancy whereas, no elevation will be observed in pseudopregnant tigress. Therefore, from the investigation it is concluded that the pseudopregnancy could be differentiated on the basis of hormone concentration along with the behavioural changes in the pregnant animal. Relaxin and progesterone both as early as 49 days after mating, whereas, PGFM elevation and peak at around 63 days after mating could differentiate between true pregnancy and pseudopregnancy.

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Ethics declaration

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.

Conflict of interest

The authors declare that they have no conflict of interest.

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