Research Article:

MONITORING PREGNANCY STATUS IN CAPTIVE ASIAN ELEPHANTS *(ELEPHAS MAXIMUS) OF* SAURAHA, NEPAL BASED ON BLOOD PROGESTERONE PROFILE

Grihamani Nepal[a*], Gokarna Gautam[a], Kamal Prasad Gairhe[b], Amir Sadaula[c], Chet Raj Pathak[a] and Bhuminand Devkota[a]

[a]Faculty of Animal Science, Veterinary Science and Fisheries, Agriculture and Forestry University, Rampur, Chitwan

[b]Chitwan National Park, Sauraha, Chitwan, Nepal

[c] National Trust for Nature Conservation, Sauraha, Chitwan, Nepal

[*]Corresponding author: gmnepalvet@gmail.com

Received date: 10 April 2023, Accepted date: 17 March 2025

DOI: https://doi.org/10.3126/jafu.v6i1.79089

ABSTRACT

Progesterone is the major reproductive hormone in female elephants to maintain pregnancy and is produced by the corpus luteum (CL) and placenta. This study routinely monitored the pregnancy status of captive Asian elephants (*Elephas maximus*) of Sauraha, Nepal, based on individual blood progesterone profiles. The five captive Asian elephants were selected from the Elephant Breeding Center (EBC) and the National Trust for Nature Conservation (NTNC). A purposive sampling technique was adopted to collect blood samples at an interval of a month for 16 months from the ear vein using a vacutainer set with 18-gauge needles. Blood progesterone concentrations were measured from blood serum samples using an enzyme-linked immunosorbent assay (ELISA). The serum progesterone concentration in non-pregnant elephants ranged from 57.01 pg/ml to 123.10 pg/ml (an average: 93.27±6.98 pg/ml) and in pregnant elephants (89.94-340.90) pg/ml, but at mid-gestation, its minimum concentration was 250 pg/ml. In this study, in pregnant elephants, the trend of serum progesterone concentration peaked at mid-gestation and became lowest at late gestation was observed. These findings in disparities in the concentration of serum progesterone levels between non-pregnant and pregnant elephants, with the help of ELISA, could be an avenue in the prediction of pregnancy status in Asian elephants.

Key words: Breeding, captive Asian elephants, estrus, pregnancy diagnosis, serology

INTRODUCTION

Elephants are the largest terrestrial mammals, and two prominent species: the Asian elephant (*Elephas maximus*) and the African elephant (*Loxodonta africana*). The Asian elephant (*Elephas maximus*) is documented as endangered on the International Union for Conservation of Nature (IUCN) Red List of Endangered Species (Choudhury et al., 2008) and Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2017). In the Nepalese scenario, Asian elephants are present in the wild or captive states and are monitored by the government or local people (Shrestha & Shrestha, 2021). Before the last five decades, captive elephants were not raised for breeding in Nepal. However, with the increase in tourism and recreational purposes, elephants are now kept in captivity and are becoming an important source of income for local people. These elephants are constantly used in transportation, patrolling, wildlife census, and treatment of other wild animal species (Gautum & Koju, 2022) Koju NP. 2022. Demographic and health status of captive elephants around Chitwan National Park, Nepal. Biodiversitas 23: 1621-1627. Elephants have been captive from ancient times. As a

keystone species, it requires proper care and management to sustain its viable population in the wild as well as a captive. However, there is a lack of study on captive elephants in Nepal. This study aimed to explore the status of captive elephants in Sauraha, Chitwan National Park, the top tourist destination of Nepal. Field survey, Key-Informant Interview (KII. In this scenario, the Government of Nepal established an Elephant Breeding Center (EBC) at Khorsor, Sauraha near Chitwan National Park (CNP) in 1986 to retain the traditional skills of training elephants and their handlers (Gairhe, 2012). The elephants of the EBC are used solely for captive breeding and infrequently for ecotourism, wildlife research, conservation, education, and ceremonies (Yakubu et al., 2016). Considering the huge cultural and economic value of Asian elephants, it is important to understand their habitat ecology, reproductive status, breeding patterns, and many other aspects for their sustainable conservation strategy to increase their overall value in the future. The major reproductive hormones in female elephants, like estrogen and progesterone, are important and have crucial physiological characteristics to display their dynamics. However, to monitor overall reproductive status as well as detect and confirm pregnancy, a non-invasive technique of monitoring progesterone profile in the blood circulation is widely used in domestic as well as wild animals, including elephants (Brown & Lehnhardt, 1995; Kajaysri & Nokkaew, 2014). This technique has a high merit in wild animals due to its applicability and accuracy.

In the Nepalese captive Asian elephants, some studies have been conducted on conservation, disease surveillance, management, and census. However, there are limited studies related to elephant reproduction and breeding. In most cases, monitoring of reproductive status relies on the experience of elephant keepers (mahouts) at the EBC, who possess traditional knowledge. Nonetheless, accurately determining the gestation period, detecting heat, and recognizing pregnancy symptoms, even in the late stages, can be challenging for them to take the necessary actions. Early detection of pregnancy and understanding reproductive status are crucial in captive elephants from a management perspective (nutritional and physical work or rest). Establishing a precise technique for confirming pregnancy is vital among female elephants. Furthermore, routine endocrine monitoring, the focus of this research, serves as a valuable tool for making decisions about the reproductive management of elephants. Although monitoring progesterone profiles from blood is a straightforward, easy, and accurate technique for detecting the reproductive status of elephants, its application and validation are minimal in other Asian elephant habitat countries like Nepal.

For the first time, this study aimed to monitor the pregnancy status of captive Asian elephants (*Elephas maximus*) in Sauraha, Nepal, based on their blood progesterone profiles. In addition, this study highlights the pattern of changes in each elephant across various experimental groups, focusing on alterations in the level of circulating progesterone in the blood during different stages of pregnancy.

MATERIALS AND METHODS

Study area and experimental animals

The study area included the elephant breeding center (EBC), with global positioning system (GPS) coordinates (27.58005°N, 84.49294°E), and the National Trust for Nature Conservation (NTNC) in Sauraha, which has GPS coordinates (27.57382°N, 84.49891°E). Five mature captive Asian elephants were randomly selected from a group, four from the EBC and one from the NTNC. The selected elephants had a recent mating history, but no reproductive problems were recorded. The study period lasted for 16 months, beginning in February 2018. The selected elephants were categorized based on their pregnancy status: negative control (no mating or calving history), positive control (mating history with confirmed symptomatic pregnancy), and experimental (mating history but not confirmed symptomatic pregnancy) (Table 1).

Table 1. Description and list of elephants in the pregnancy monitoring study

Elephant (Name)	Age (Years)	Previous calving (Times)	Mating history (Date)	Elephant (Grouping)
Jun kali	49	0	No	NC
Loktantra kali	12	0	May, 2017	PC
Aishwarya mala	40	3	Dec, 2017	Ex
Karnali kali	20	2	Jan, 2017	Ex
Chitwan kali	31	3	Jan, 2018	Ex

NC, Negative Control; PC, Positive Control; Ex, Experiment

General nutritional, health, and reproductive management of elephants

The elephants were managed by mahouts who used to take them inside the park in the daytime (9:00 am to 3:00 pm) every day for foraging. The kuchi (rice grain, molasses, and salt wrapped in grass bands) was an additional supplement to the elephants, provided to them in the evening. The elephants were managed with a deworming schedule biannually and inspected regularly by a veterinarian from the government and NTNC. Mostly, the wild male elephants named Ronaldo, Dhurbe, and Govinde entered the EBC to detect the estrus period of the female elephants, and during the standing heat, they mate.

Blood sample collection

A total of 5 ml of blood sample was collected from the ear vein of each animal monthly. Keeping the tubes at 4°C in a cool box with ice packs, transported to a molecular laboratory in NTNC, Sauraha, and centrifuged for 5 min at 1500 rpm for the separation of serum. The serum sample was kept in the Eppendorf tubes and stored at −20°C until hormone analysis.

Progesterone hormone analysis

Serum progesterone was analyzed by enzyme-linked immunosorbent assay (ELISA) (Kit, Cat. N. KA0299, Abnova, Taiwan) following the manufacturer's protocol. The concentration of serum progesterone was calculated and detected after being examined with an ELISA plate reader (BioTek®, USA).

Statistical analysis

The data entry, analysis, and representations were performed in Microsoft Excel 2010.

RESULTS AND DISCUSSION

Changing pattern of progesterone in elephants under study

Progesterone pattern of negative control (Jun kali)

The highest and lowest values of the progesterone hormone during the sixteen months were 123.10 pg/ml (10th month of sampling) and 57.01 pg/ml (7th month of sampling), respectively, with an average of 93.27±6.98 pg/ml (mean ± SD) (Fig. 1). It indicates that the chance of pregnancy is rare if the progesterone level is less than 100 pg/ml in the Asian elephants.

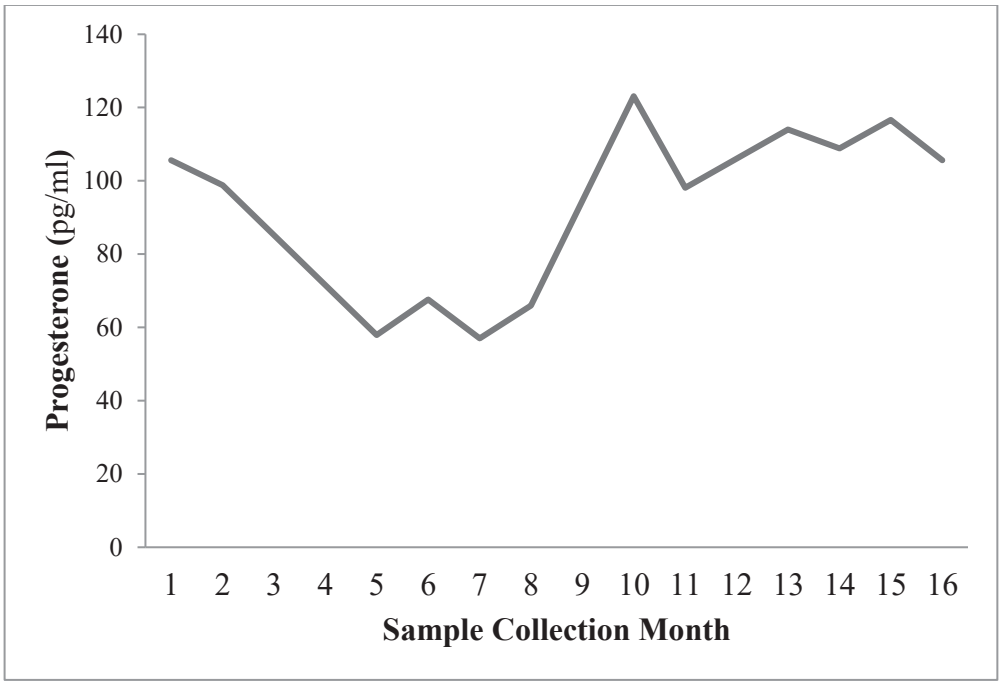

Fig. 1 Progesterone pattern of elephant Jun kali.

Progesterone pattern of positive control (Loktantra kali)

This elephant was conceived for the first time at 12 years of age. Initial pregnancy confirmation was tentative, based on physical changes, such as abdominal and mammary gland enlargement. The peak progesterone level (249.60 pg/ml) was recorded in the 1st month of sampling, corresponding to 9 months of pregnancy (mid-gestation), while the lowest level (106.40 pg/ml) was observed at parturition in the 14th month of sampling (22 months of gestation) (Fig. 2).

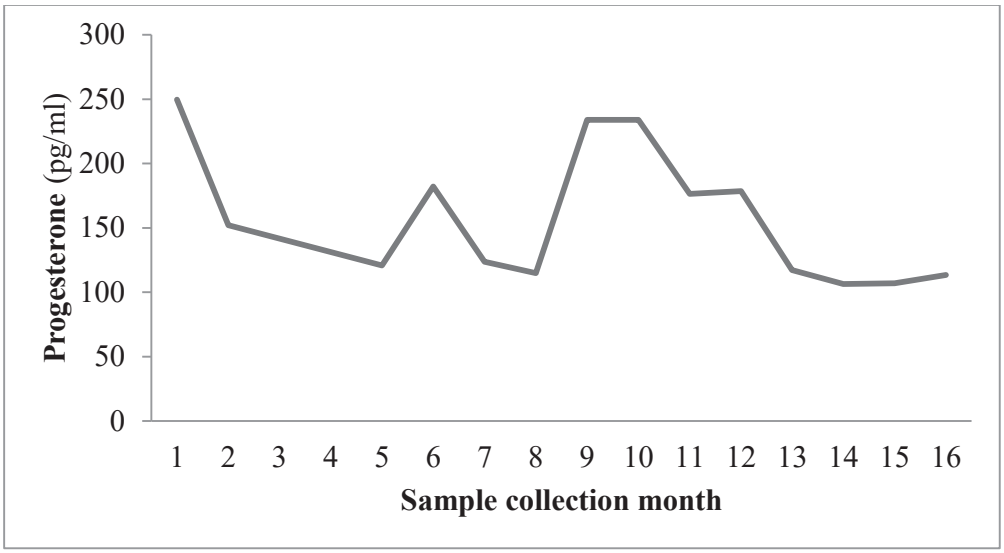

Fig. 2 Progesterone pattern of elephant Loktantra kali.

Progesterone pattern of Aishwarya mala

The highest progesterone level was 208.00 pg/ml (6th month of sampling), and the lowest was 73.37 pg/ml (8th month of sampling) (Fig. 3). A similar pattern was observed with a peak during the 9th-10th months and a decline in the 11th month. The progesterone levels showed a sequential rise and fall, with two peaks occurring at 4 months (16 weeks) intervals.

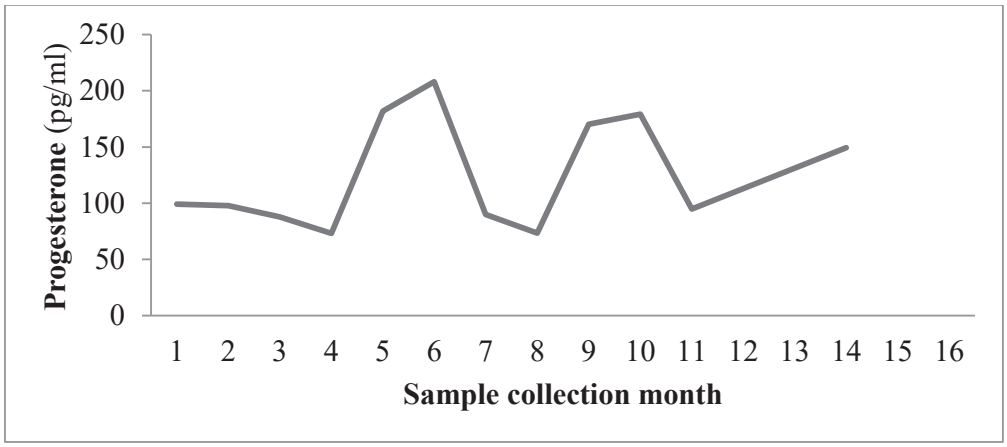

Fig. 3 Progesterone pattern of elephant Aishwarya mala

Progesterone pattern of Karnali Kali

This elephant had a history of mating and calved in the 11th month of sample collection. Retrospective calculation indicated that sampling began at the 12th month of pregnancy (Fig. 4). The highest progesterone level, 340.90 pg/ml, was recorded at mid-gestation (12th month of gestation), gradually declining to 89.94 pg/ml at term in 11 months of sampling (22nd month of gestation).

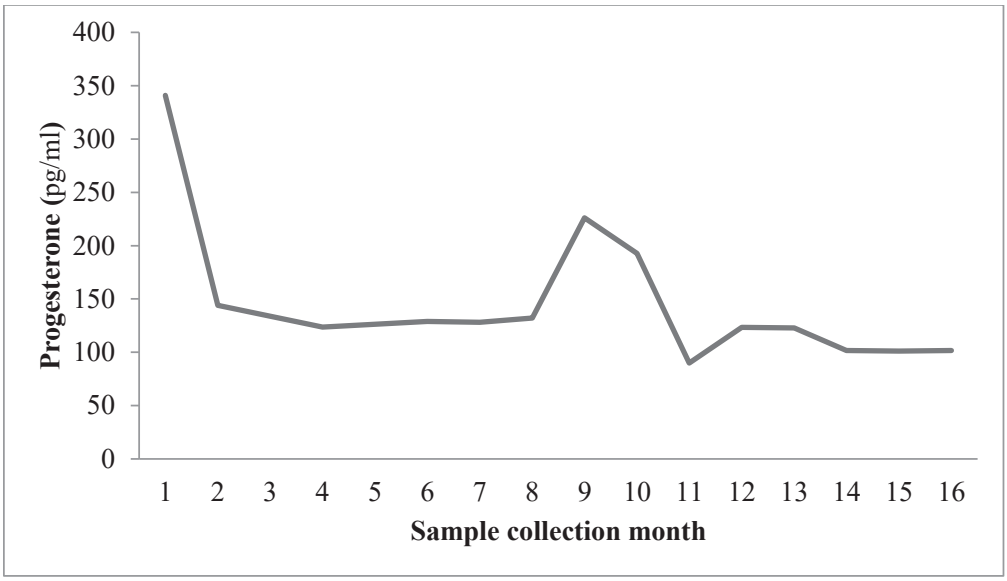

Fig. 4 Progesterone pattern of elephant Karnali kali.

Progesterone pattern of Chitwan kali

The lowest progesterone level was 82.74 pg/ml in the 3rd month, and the highest was 336.7 pg/ml in the 14th month of sampling. Despite only a recorded mating history, the cyclic rise and fall in progesterone (Fig. 5) suggests regular ovarian activity. She is likely to exhibit normal reproductive events in the future, including ovulation, mating, and successful calving.

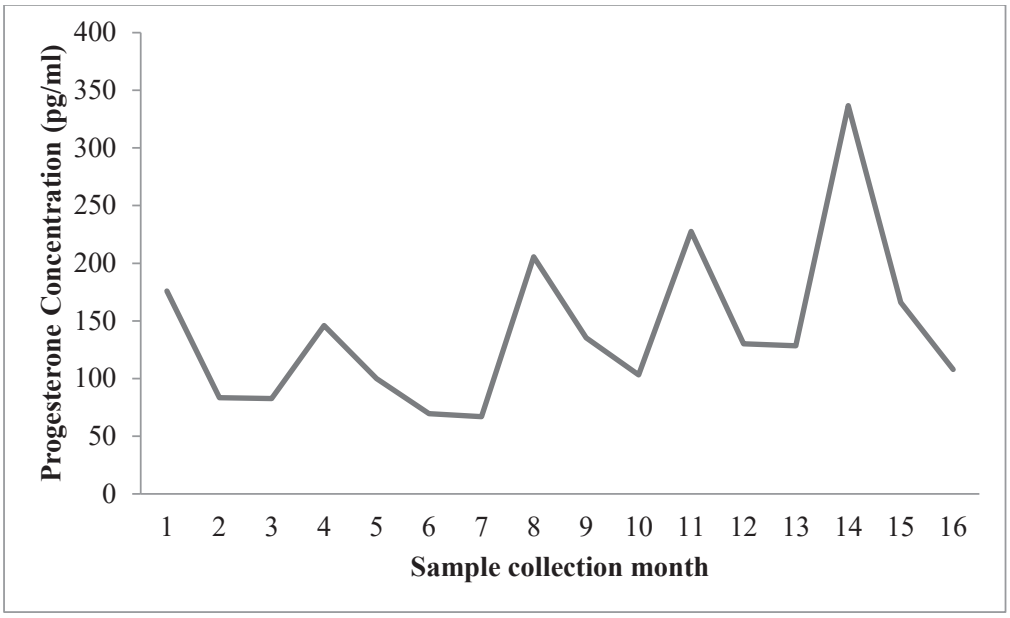

Fig. 5 Progesterone pattern of elephant Chitwan kali.

Comparative profile of the progesterone hormone

Positive Control vs Negative Control

The positive control (Loktantra kali) exhibited a clear and typical fluctuating pattern of progesterone, ranging from 106.40 to 249.60 pg/ml. In contrast, the negative control (Jun kali) showed minimal fluctuation, with levels ranging from 57.01 pg/ml to 123.10 pg/ml. The average progesterone concentration was lower in negative control (93.27±6.98 pg/ml) compared to the positive control (157.87±13.8 pg/ml) (Fig. 6).

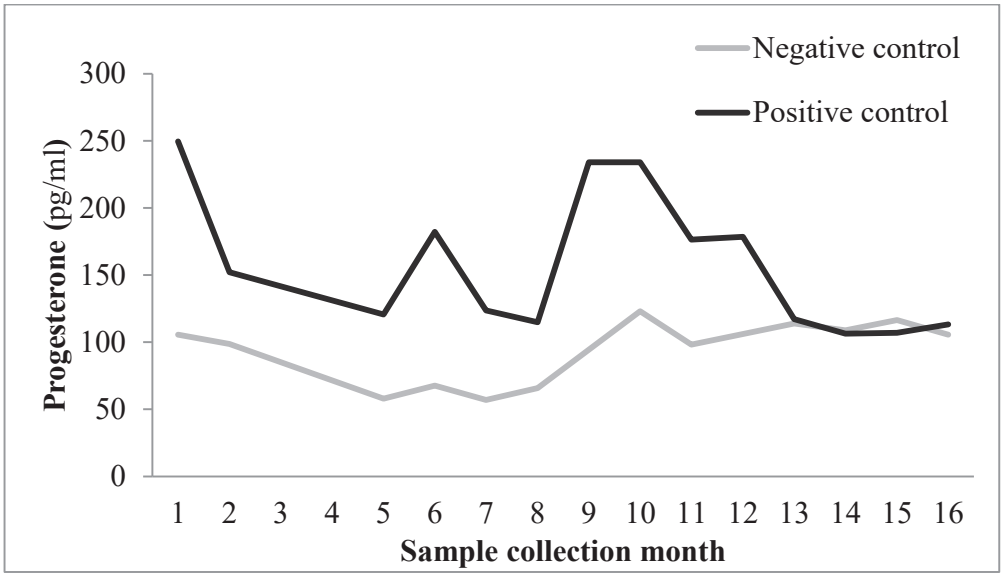

Fig. 6 Comparison between progesterone levels in positive and negative controls.

Comparison between pregnant elephants with estimated similar months of pregnancy

In this study, Karnali kali calved three months earlier than Loktantra kali; therefore, progesterone values were compared for the corresponding months of the 22-month pregnancy every month. The peak progesterone levels were observed during mid-gestation, reaching 249.60 pg/ml in the 9th month (Loktantra kali) and 340.90 pg/ml in the 12th month (Karnali kali). Subsequently, the concentrations declined until parturition, with the lowest 106.40 pg/ml and 89.94 pg/ml,

respectively (Fig. 7). However, no elephants were found in early gestation during the study period for the monitoring of progesterone.

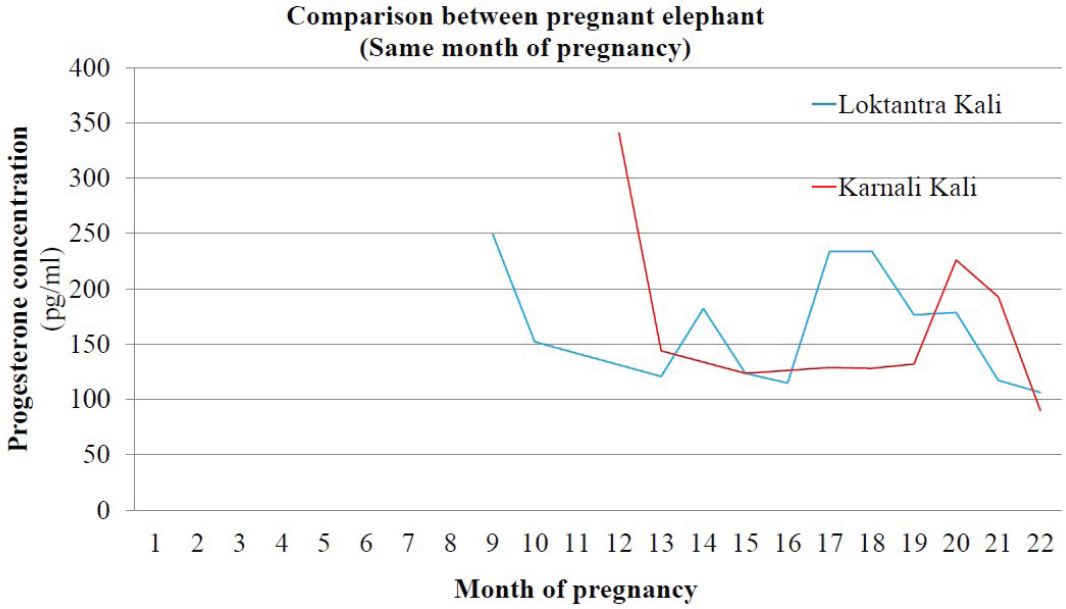

Fig. 7 Comparison between pregnant elephants at the same stage of pregnancy.

Through monitoring blood progesterone profiles, this research assessed the gestational status of the captive Asian elephants at Sauraha, Nepal. This study is the first of its kind conducted for captive Asian elephants in Nepal. The analysis showed that there was a reliable reflection of progesterone levels across different stages of pregnancy, which could aid in pregnancy detection and monitoring for all stages of pregnancy. Differences in age and previous pregnancies among individuals are likely to be the reason for differences in progesterone hormone levels during pregnancy.

In this study, progesterone levels in non-pregnant elephants (Jun kali) ranged from 57.01 to 123.10 pg/ml, with an average of 93.27±6.98 pg/ml. These findings are similar to previous studies conducted in the United States, where serum progesterone during the non-luteal phase of estrus ranged from undetectable to 100 pg/ml, with a mean of 53±10.7 pg/ml (Brown, 2006; Oliveira et al., 2008). Additionally, similar progesterone levels in non-pregnant Asian elephants are found by Sukumar et al. (1991). The monitored progesterone concentrations suggest an absence of regular estrous cycle in our study.

We found the progesterone levels ranged from 89.94 to 349.60 pg/ml during pregnancy, with peak levels at mid-gestation (9-12 months) and the lowest at parturition. These results align with the previous studies conducted on the progesterone profile in Asian elephants. A decline in serum progesterone during the final month of gestation was found by Brown and Lehnhardt (1995) in Srilanka while Kajaysri and Nokkaew (2014) observed a sharp decrease in serum progesterone at the final day of labor. Harris et al. (2009) studied changes in progesterone levels throughout gestation, noting peak values during mid-pregnancy along with flare changes during the period. Another study by Brown et al. (2000) also reported the same patterns of progesterone in pregnant Asian elephants.

The elephants Aishwarya Mala and Chitwan Kali did not exhibit excessive progesterone levels sufficient to maintain pregnancy, but both showed fluctuations every 4 months (16 weeks). Such fluctuations are typically observed in cyclic elephants. A similar pattern was reported by Oliveira

et al. (2008) in the USA, where progesterone levels in cyclic elephants fluctuated during both the luteal and non-luteal phases of the estrous cycle. In addition, Sukumar et al. (1991) observed similar cyclic fluctuations in progesterone in Asian elephants during their estrous cycle in India. Similarly, Brown et al. (2000) found fluctuating progesterone levels in captive elephants, which were indicative of regular cyclicity.

CONCLUSION

The serum progesterone level differed greatly between non-pregnant and pregnant elephants. In pregnant elephants, serum progesterone concentration peaks (>250 pg/ml) at mid- and is lowest at late gestation. Among the pregnant elephants, the variations may exist individually in serum progesterone level and its pattern. Thus, in elephants, at least in the captive state, monitoring the progesterone level is crucial for the detection physiological status of females for further managemental planning.

ACKNOWLEDGEMENTS

The authors would like to acknowledge all the staff and mahouts from EBC and NTNC who supported them during the field work. Also, thankful to Mr. Kiran Raj Rijal, Mr. Prushottam Pandey, and Mr. Janardan Dev Joshi for their help and support during sample collection and laboratory work.

ETHICAL APPROVAL

The ethical approval for these experiments was obtained by the Department of National Parks and Wildlife Conservation (DNPWC).

REFERENCES

Brown, J. L. (2006). Reproductive endocrinology. In M. E. Fowler & S. K. Mikota (Eds.), *Biology, medicine, and surgery of elephants* (1st ed., pp. 377-388). Ames, IA: Blackwell Publishing.

Brown, J. L., Walker, S. L., & Moeller, T. (2004). Comparative endocrinology of cycling and non-cycling Asian (Elephas maximus) and African (*Loxodonta africana*) elephants. *General and Comparative Endocrinology*, 136(3), 360-370.

Brown, J. L., & Lehnhardt, J. (1995). Serum and urinary hormones during pregnancy and the peri- and postpartum period in an Asian elephant (Elephas maximus). *Zoo Biology*, 14(6), 555–564. https://doi.org/10.1002/zoo.1430140608.

Choudhury, A., Lahiri Choudhury, D. K., Desai, A., Duckworth, J. W., Easa, P. S., Johnsingh, A. J. T., …Wikramanayake, E. (2008). Elephas maximus [WWW document]. *IUCN Red List Threat. Species*. https://doi.org/10.2305/IUCN.UK.2008.RLTS.T7140A12828813.en

CITES. (2017). *Appendices I, II, and III, Convention on international trade in endangered species of wild fauna and flora*.

Gairhe, K. P. (2012). Veterinary care and breeding of elephants in Nepal. *Gajah*, 37(2012), 27–30. https://www.researchgate.net/publication/282567392

Gautum, S., & Koju, N. P. (2022). Demographic and health status of captive elephants around Chitwan National Park, Nepal. *Biodiversitas*, 23(3), 1621–1627. https://doi.org/10.13057/biodiv/d230353

Harris, M., Brown, J. L., & Lehnhardt, J. (2009). Species and fetal gender effects on the endocrinology of pregnancy in elephants. *General and Comparative Endocrinology*, 162(2), 180–185. https://doi.org/10.1016/j.ygcen.2009.02.008

Kajaysri, J., & Nokkaew, W. (2014). Assessment of pregnancy status of Asian elephants (Elephas maximus) by measurement of progestagen and glucocorticoid and their metabolite concentrations in serum and feces, using enzyme immunoassay (EIA). *Journal of Veterinary Medical Science*, 76(3), 363–368. https://doi.org/10.1292/jvms.13-0103

Oliveira, C. A., Felippe, E. C. G., & Chelini, M. O. M. (2008). Serum cortisol and progestin concentrations in pregnant and non-pregnant Asian elephants (Elephas maximus). *Research in Veterinary Science*, 84(3), 361–363. https://doi.org/10.1016/j.rvsc.2007.05.009

Shrestha, S., & Shrestha, J. (2021). Asian elephants and their status in Nepal: A review. *Journal of Agriculture and Natural Resources*, 4(2), 227–237. https://doi.org/10.3126/janr.v4i2.33828

Sukumar, R., Krishnamurthy, V., Wemmer, C., & Rodden, M. (1991). Demography of captive Asian elephants (Elephas maximus) in southern India. *Zoo Biology*, 10(4), 389–398. https://doi.org/10.1002/zoo.1430100406

Yakubu, Y., Ong, B. L., Zakaria, Z., Hassan, L., Mutalib, A. R., Ngeow, Y. F., Verasahib, K., & Razak, M. F. A. A. (2016). Evidence and potential risk factors of tuberculosis among captive Asian elephants and wildlife staff in Peninsular Malaysia. *Preventive Veterinary Medicine*, 125, 147–153. https://doi.org/10.1016/j.prevetmed.2016.01.008