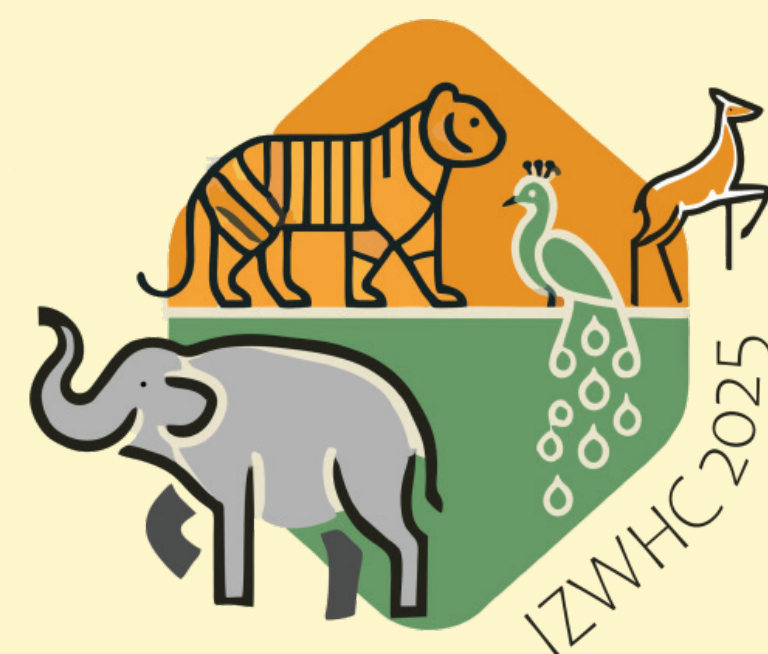




Correlation of Vaginal Cytology, Estrous Behaviour and Faecal Hormone for Assessment of Reproductive Health Status in Captive Common Leopard (*Panthera pardus fusca*).



Dr. Ruchika Sangale^{a*}, Dr. Ajay Deshmukh^a, Dr. Nikorn Thongthip^a, Dr. Ankush Dubey^a, Shubham Chhapekar^a

Greens Zoological Rescue and Rehabilitation Centre, office SSO, Annexe-5, at post-R Greens, Village - Motikhavdi dist. Jamnagar - 361142, Gujarat, India

INTRODUCTION:

The conservation of wild felids is a pressing concern, with most species threatened with extinction (IUCN, 2019). Understanding their reproductive physiology is crucial for developing effective ex-situ conservation programs. Felids are induced ovulators, and their reproductive cycles are characterized by alternating periods of Oestrus and inter-Oestrus. In captivity, without mating and conception female leopards often experience uninterrupted Oestrus cycles, leading to chronic stress, hormonal imbalances, reproductive health issues such as cystic ovaries, and cystic endometrial hyperplasia which is often overlooked in zoo management (Johnston et al. a2001).

This study focused on five adult captive common leopard (*Panthera pardus fusca*) females presented with h/o rolling, lordosis, rubbing and slight thin transparent vaginal discharge for a period longer than Oestrus phase and can be indicator of reproductive health issues. The early diagnosis and management of the conditions is crucial to save the fertility; Hence, vaginal cytology evaluation was used to monitor fluctuations in estrogen concentrations and diagnose reproductive health status. This technique has proven reliable in domestic species, but its application in wild felids is limited. This case study aimed to investigate the efficacy of vaginal cytology assessment, combined with behaviour observation and fecal hormone analysis, in predicting normal and unusual Oestrus cycles in common leopards. By exploring the correlation between these parameters, we hope to improve reproductive health monitoring and management in captive wild felids.

MATERIALS AND METHODS:

Vaginal cytology sampling and evaluation:

Sixty vaginal samples were collected thrice a week for 1.5 month from five common leopard females during positive reinforcement training. Samples were collected using cotton-tipped swabs gently introduced about 3 cm dorsally into vagina and rotated against vaginal wall, rolled onto glass slides, dried, fixed, and stained using field stain. Evaluation at 10x magnification assessed mucosal epithelial cells, neutrophils, bacterial cells, and debris. Cytology patterns were used to determine estrous phase (superficial anucleate cells, absence of neutrophils, presence of bacteria) or diestrus phase (parabasal/intermediate cells, presence of neutrophils and debris) (Callealta et al. 2018) (Johnston et al. b2001; Mills et al.1979).



Fig.1 Vaginal swab sample collection.

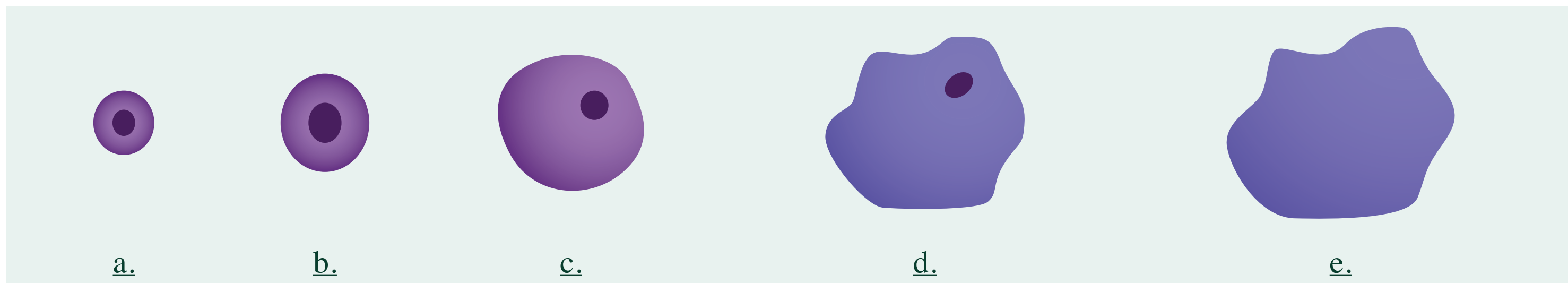


Fig.1 Diagram of epithelial cells that observe in a vaginal smear. a) Basal cell; b) Parabasal cell; c) Intermediate cell; d) Superficial nucleated cell; e) Superficial anucleated cell

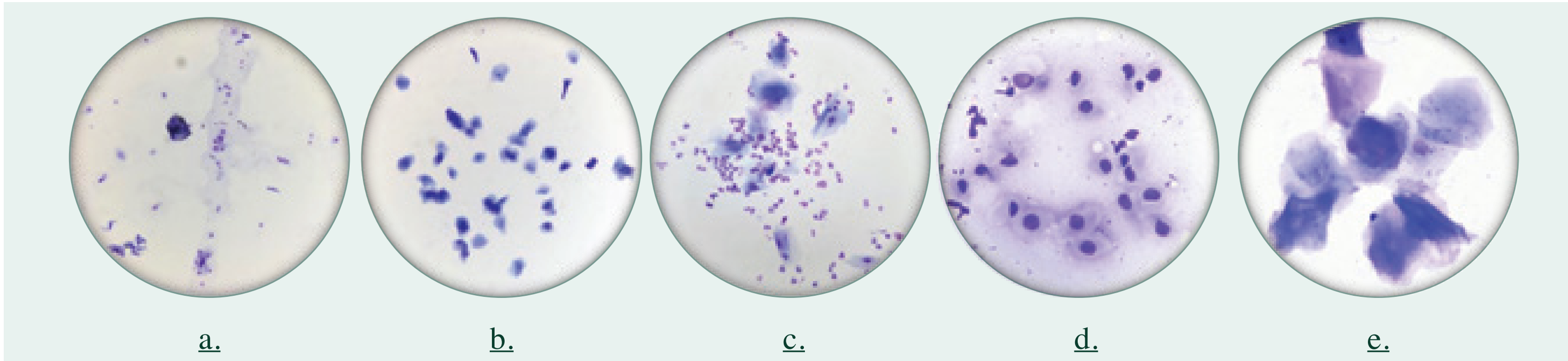


Fig.2 Vaginal smear collected from Common leopard during a) Dioestrus; b) Oestrus; c) Inter-oestrus; d) shows in detail clump of parabasal cells and a few neutrophils surrounded by vaginal mucous. e) shows in detail nucleated and anucleated superficial cells associated with bacteria (also noticeable in b)

Table.1 Means and ranges of percentage of epithelial cells and neutrophils found in different stages of common leopard Oestrus cycle					
Vaginal Epithelial Cells	Pro-oestrus	Oestrus	Inter-oestrus	Dioestrus	Anoestrus
Parabasal cells	18% (0-34)	0.3% (0-3)	8.9% (0-29)	48% (10-85)	9.7% (0-50)
Intermediate cells	60% (57-67)	11% (0-25)	75.5% (50-100)	50% (10-85)	87.4% (50-100)
Superficial nucleated cells	20% (6-40)	24% (3-42)	13.2% (0-41)	2% (0-5)	2.7% (0-22)
Superficial anucleated cells	2% (0-6)	63.6% (30-95.6)	1.9% (0-3)	0%	0.2% (0-2)
Neutrophils (per 100 epithelial cells)	0-10%	4.7 (0-10)	32% (1-78)	32% (0-97)	3% (0-50)
Normal cyclic leopard Oestrus cycle	2-4 days	6-9 days		21-28 days	lactational anoestrus post-parturition 21-25 days

Behavioral Monitoring:

For 1.5 month, the behavior of five females was monitored twice a day (at sunrise and dusk) 5-7 days per week, in session of 15-60 min. a relative increase in frequency of specific reproductive symptoms such as purring, flirting run, lordosis, allowing for mounting, and rolling enabled detection of females in natural Oestrus (Stanton et al., 2015).



Fig.3 Female Leopard exhibiting rolling behaviour

Collection of fecal samples for hormone estimation:

Total 60 fecal samples were collected thrice a week for 1.5-month duration by animal care staff for fecal progesterone and estrogen analysis. Samples were placed in labeled plastic bags and stored at -20°C until processing. Later, fecal samples were lyophilized overnight, crush into fine powder and thoroughly mixed. 0.25 gm of each lyophilized sample was extracted by mixing with 2.5 ml 90% ethanol overnight on a mechanical rocker. The extracted samples centrifuged (1000x g, 10 min), and supernatants were removed and metabolite of hormone progesterone and estrogen were analyzed with DetectX progesterone & estrogen ELISA kit by arbor assays, USA.

RESULT AND DISCUSSION

Table.2 Vaginal cytology findings throughout oestrus cycle of five common leopard female											
DATE	03/04/24	06/04/24	10/04/24	13/04/24	17/04/24	20/04/24	24/04/24	27/04/24	01/05/24	04/05/24	08/05/24
SARA											
Parabasal cells (%)	60-70	50-60	40-50	30	20	7%	0	0	13	36	52
Intermediate cells (%)	20	20-30	30-40	40-50	30	10-20	10-20	6	62	51	
Superficial nucleated cells (%)	10	10	10	20	40-50	60	30	5	18.6	10	12
Superficial anucleated cells (%)	0	0	0	0	5	10	60	89	6.4	3	0
Neutrophils (/100 epithelial cells)	0	0.4	1	0	0	2-3	0	4	41	21	2
Phase	Dioestrus	Dioestrus	Dioestrus	Dioestrus	Prooestrus	Prooestrus	Oestrus	Oestrus	Interestrus	Dioestrus	Dioestrus
Behavior	Normal	Normal	Normal	Normal	Normal	Normal	Rolling	Rolling	Normal	Normal	Normal
Impression:	Based on the ratio of superficial: parabasal cells, during sampling period observed female transitioning from luteal to follicular phase. (Short Luteal Phase)										
VIJAYA											
Parabasal cells (%)	0	15	10	40-50	70	60-65	60-65	20	13	0	0
Intermediate cells (%)	0	60	20-30	30	20	30	20-30	30	62	0	0
Superficial nucleated cells (%)	30	19	50	10	10	3	15	40-50	18.6	30	20
Superficial anucleated cells (%)	70	6	10	2	0	0	0	5	6.4	70	80
Neutrophils (/100 epithelial cells)	0	42	1	8	5	0	5	0	6.4	0	2
Phase	Oestrus	Interestrus	Dioestrus	Dioestrus	Dioestrus	Dioestrus	Dioestrus	Prooestrus	Prooestrus	Oestrus	Oestrus
Behavior	Rolling	Normal	Normal	Normal	Normal	Normal	Normal	Rolling	Rolling	Rolling	Rolling
Impression:	Based on the ratio of superficial: parabasal cells, during sampling period observed female transitioning from luteal to follicular phase.(Short Luteal Phase)										
SHANTI											
Parabasal cells (%)	70	60	40-50	50	50-60	60	70	65	72	89	63
Intermediate cells (%)	25	30	15-20	20	30	20	30	35	25	8	34
Superficial nucleated cells (%)	0	9%	20	30	10	20	0	0	3	3	3
Superficial anucleated cells (%)	0	0	0	0	0	0	0	0	0	0	0
Neutrophils (/100 epithelial cells)	5	9	0	0	0	0	0	0	0	0	0
Phase	Dioestrus	Dioestrus	Dioestrus	Dioestrus	Dioestrus	Dioestrus	Dioestrus	Dioestrus	Dioestrus	Dioestrus	Dioestrus
Behavior	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Impression:	Based on the ratio of superficial : parabasal cells, during sampling period observed female was in luteal phase of Oestrus cycle.										
NIMMIE											
Parabasal cells (%)	0	0	0	0	0	0	0	0	0	0	0
Intermediate cells (%)	0	0	0	0	0	0	0	0	0	0	0
Superficial nucleated cells (%)	20	18	10	0	0	0	0	7	5	0	0
Superficial anucleated cells (%)	80	82	90	100	100	100	100	93	95	100	100
Neutrophils (/100 epithelial cells)	6	2	0	0	0	0	0	2	8	5	0
Phase	Oestrus	Oestrus	Oestrus	Oestrus	Oestrus	Oestrus	Oestrus	Oestrus	Oestrus	Oestrus	Oestrus
Behavior	Rolling	Rolling	Rolling	Rolling	Rolling	Rolling	Rolling	Rolling	Rolling	Rolling	Rolling
Impression:	Based on the ratio of superficial : parabasal cells, during sampling period observed female was in follicular phase of Oestrus cycle. (Normal Oestrus length: 5-8 days) suspected for follicular cyst/ polycystic ovaries.										
WINNIE											
Parabasal cells (%)	0	0	0	0	0	0	0	0	0	0	0
Intermediate cells (%)	0	0	0	0	0	0	0	0	0	0	0
Superficial nucleated cells (%)	10	2-5	20	0	20-30	0	5	7	5	0	0
Superficial anucleated cells (%)	90	90-95	70-80	100	70	100	80-90	93	95	100	100
Neutrophils (/100 epithelial cells)	6	0	0	0	0.5	0	5	2	8	5	0
Phase	Oestrus	Oestrus	Oestrus	Oestrus	Oestrus	Oestrus	Oestrus	Oestrus	Oestrus	Oestrus	Oestrus
Behavior	Rolling	Rolling	Rolling	Rolling	Rolling	Rolling	Rolling	Rolling	Rolling	Rolling	Rolling
Impression:	Based on the ratio of superficial : parabasal cells, during sampling period observed female was in follicular phase of Oestrus cycle. (Normal Oestrus length: 5-8 days) suspected for follicular cyst/ polycystic ovaries.										

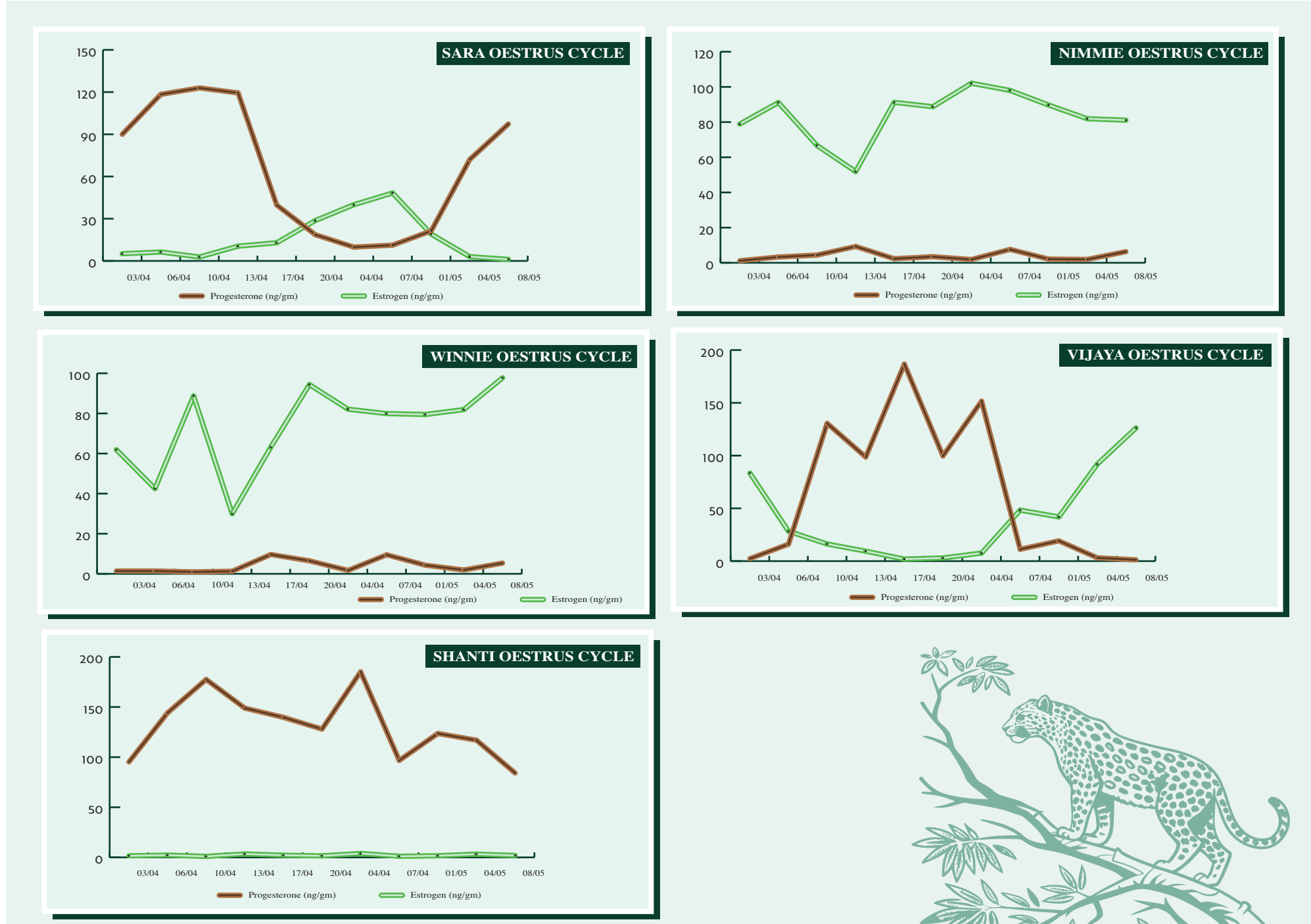


Fig.4 Fecal progesterone and estrogen metabolite profile in five common leopard

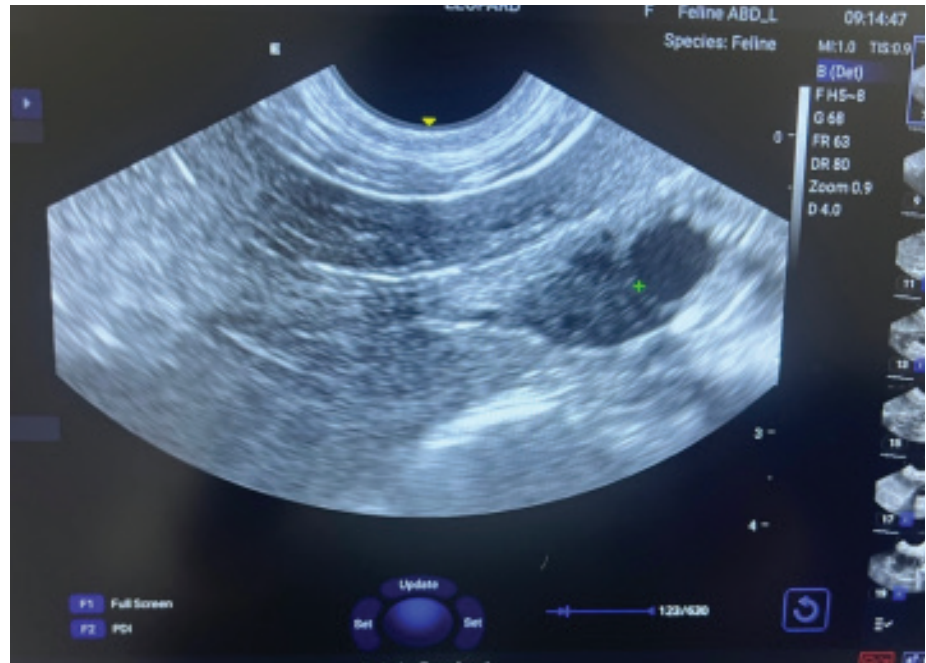


Fig.5 USG of Winnie showed a dominant and multiple small follicle on ovary.

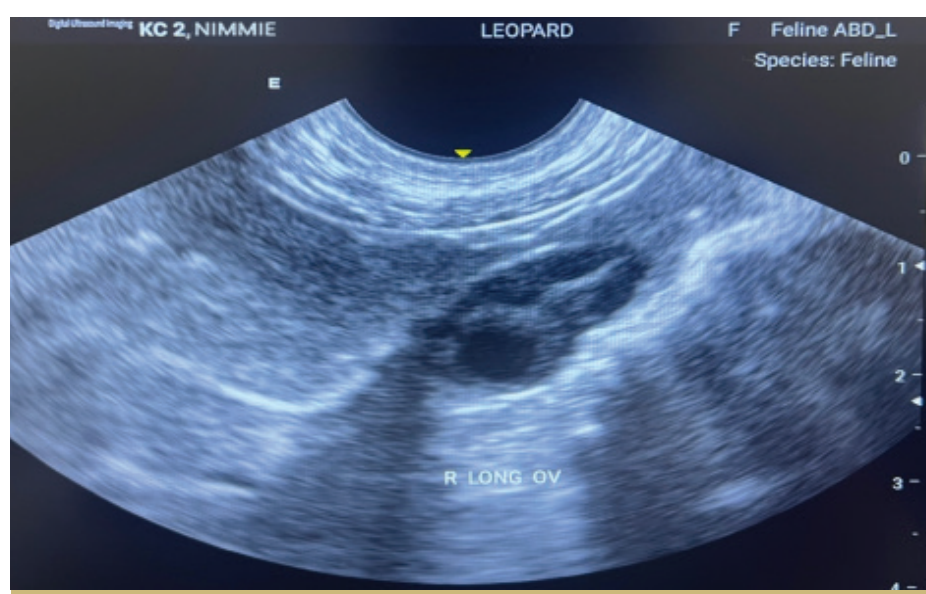


Fig.6 USG of Nimmie showed follicular cyst on ovary.

CONCLUSION:

The vaginal cytology evaluation, after correlating with estrous-related behavior and fecal hormone monitoring, proved to be effective technique and can be applied for early diagnosing reproductive health related issues to troubleshoot them on time in captive wild felids and conserve fertility.

REFERENCE

- Callealta, I., Ganswindt, A., Goncalves, S., Mathew, A., & Lueders, I. (2018). Detection of Simonsiella spp. in the Vagina of Lions and Leopard in Oestrus. Reproduction in Domestic Animals, 53(6), 1605-1608.
- Johnston, S. D., Root Kustritz, M. V., & Olson, P. N. (a2001). The feline oestrus cycle. In: Canine and feline theriogenology. Philadelphia: WB Saunders Co, 396.
- Johnston, S. D., Root Kustritz, M. V., & Olson, P. N. (b2001). Vaginal cytology. In: Canine and feline theriogenology. Philadelphia: WB Saunders Co, 32.
- Mills, J. N., Valli, V. E., & Lumsden, J. H. (1979). Cyclical changes of vaginal cytology in the cat. The Canadian Veterinary Journal, 20(4), 95.
- Stanton, L.A., Sullivan, M.S., Fazio, J.M., 2015. A standardized ethogram for the Felidae: a tool for behavioral researchers. Appl. Anim. Behav. Sci. 173, 3–16. <https://doi.org/10.1016/j.applanim.2015.04.001>.

ACKNOWLEDGEMENT

We are extremely grateful to Gujrat Forest Department, Sh. Vivan Karani (CEO), Sh.Aakash Pratapsingh (Head operations), Dr. Brij Kishore Gupta (Director), Dr. Anil Sharma (Lab. HOD), Sh.Akshay Shah (Cluster head), Sh. Neeraj Sangwan (Cluster head), Sh.Naman Dani (Facility manager) for all the invaluable support and encouragement.