

SHORT COMMUNICATION

Post partum reproductive assessment in lowland Tapir (*Tapirus terrestris*): a case report

Avaliação reprodutiva pós-parto em Tapir (*Tapirus terrestris*): relato de caso

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SUMMARY

The lowland tapir is the biggest Brazilian terrestrial mammal, which belongs to the order Perissodactyla, suborder Hippomorpha, superfamily Tapiroidea and a member of the family Tapiridae. At tropical forest the tapir is involved with seed dispersal. The knowledge of this wild animal reproductive cycle is one way to help its preservation. The stress due to restrain of captive or free-ranging wild animal in order to sample collection limits endocrine study once it can be hazard for the estrous cycle. One possibility is to quantify gonadal hormones at the excreta. Progesterone milk levels were studied in a tapir housed at the Araçatuba Zoo, in São Paulo, Brazil. Milk samples, vaginal cytology and rectal temperature were collected during lactation. The progesterone was quantified by radioimmunoassay solid phase (Coat-a-Count, DPC®). The standard was supplied by CENA-FAO and the assay showed sensitivity of 1.25 nmol/L with intra-assay variation of 15.36%. During most of the lactation (November to June) the female showed no detectable levels of progesterone. After 158 days (from November to April) it was detected the first progesterone peak with 2.3 nmol/L that lasted for 5 days. The second progesterone peak of 3.54 nmol/L lasted for 23 days. The lactation ceased 74 days after the first milk progesterone surge. This animal showed a prolonged lactational anestrous period (nearly 5 months) and the return of gonadal cycle by fall suggested no positive photo-period influence. The milk progesterone quantification showed to be useful for reproductive cycle evaluation of this animal, although vaginal cytology and temperature fluctuation had no relationship with hormonal levels.

UNITERMS: *Tapirus terrestris*; Milk; Progesterone; Animal reproduction.

The lowland tapirs are mammals that belong to the order Perissodactyla. This order is divided into three families (Equidae, Rhinocerotidae and Tapiridae). The tapirs are more closely related to the horse than to the rhinoceros¹.

They are characterized by a stout, swine like body, a fused nose and an upper lip forming a short proboscis, and a blunt, stubby tail². All the American tapirs are dark brown. The Brazilian tapirs have shorter, smoother pelage, and a concave dorsal head whereas that of the Baiard's tapirs is strongly convex. All young tapirs are brown with white longitudinal banding and spotting that begins to fade and nearly disappears in a year.

The lowland tapirs consume large quantities of fruit exploiting both pulp and seeds, sometimes disperse seeds over short distance during mastication. Dispersions of intact seeds that pass through the digestive tract are frequent³.

In wild animals, assessing the reproductive status, should search protocols without chemical or physical restrain. The procedure of blood collection may result in a stress response suppressing all the physiological activities that do not result in an immediate benefit, including reproduction^{4,5}. The hormonal quantification of excreta turns possible the endocrine evaluation without blood collection. Milk progesterone of Indian cattle showed high correlation with serum progesterone⁶. Methods for monitoring reproduction have been developed to allow both captive

and free-ranging wildlife evaluation avoiding chemical immobilization or physical restrain. Generally conjugated sex steroids' metabolites are measured in the urine, whereas the unconjugates' metabolites are measured in the feces. When correctly applied, these approaches allow the assessment of reproductive function for prolonged time periods that permit the objective description of cyclic physiology process or the detection of infrequent events⁷.

The vagina mucosa is covered by an epithelium that may reflect, in some species, the estrus cycle, according to variation of gonadal estradiol and progesterone. In the mare, the squamous epithelium of the vagina mucosa undergoes rhythmic changes during the estrus cycle. There seemed to be more cornified surface cells during estrus, but prominent layers of flattened cornified cells were not seen at any stage. Similarly, vaginal smears contained few cornified cells and the vaginal smearing technique was not useful for estimating stage of cycle for mares⁸. Also for other Perissodactyla specie, the black rhinoceros, the vaginal cytology was not found to be helpful for indicating the estrous cycle stage⁹. Few researchers have described tapirs regarding reproductive cycles or breeding patterns and breeding has reported to occur randomly. Body temperature and the levels of circulating progesterone in several domestic species have been studied and suggested as possible predictors of estrus, ovulation and parturition.

In the horse no change in temperature occurred that could be utilized to predict estrus, ovulation or parturition¹⁰.

The present study was therefore designed to investigate the milk progesterone excretion through post-partum period in one female of tapir, and the possibility of a relationship of hormonal levels, vaginal cytology and rectal temperature was also studied.

Milk samples were always collected in the morning (between 7 and 8 am) centrifuged for fat separation and the serum was stored in polypropylene tubes at -20°C until hormonal quantification. Rectal temperature was taken with clinical thermometer. Smears were made using sterile swabs 20 cm length, dried and later stained with Leischman stain. They were evaluated for cellularity, major cell type, and predominant epithelial cell type. The major cell type and predominant epithelial cell type were determined by examining microscope fields (100 x) counting 100 cells and annotated the percentile of each cell type. In most cases the cell type was either degenerated neutrophil or epithelial cell. Vaginal epithelial cells were of 3 kinds, large nucleus of non-cornified cells, smaller nucleus cells with moderate amounts of cytoplasm (parabasal cells) and cornified epithelial cells.

The progesterone was quantified by a solid phase I-125 radioimmunoassay kit Coat-a-Count (DPC-USA) developed for human without previous extraction. There is a high specificity (100%) to progesterone and a cross reaction of 2,4% with 11-Deoxycortizol, 2,0% with 20, Dihidro-progesterone, 1,3% with 5 beta-pregnan-3,20-dione. We used a standard milk curve supplied by CENA-FAO. Assay sensitivity was 1,25 nmol/L and intra-assay coefficient of variation was 15,36%.

The milk radioimmunoassay showed sensitivity for progesterone detection in this specie. During the lactational period that lasts from November to June, the female didn't show detectable milk progesterone levels until 158 days after parturition. This period of lactational anestrus has been stated in the mare and may continue until the foal is weaned at four to six months. This prolonged anestrus period in the tapir may be result of the inhibitory effect of nursing on estrus and follicular development. It was showed that foal removal hastened the onset of estrus in mares reducing the interval to estrus¹¹. The lactational anovulation is the simplest fertility-restraining device using the suckling stimulus of the infant to inhibit pituitary gonadotrophin secretion, and hence suppress follicular development¹².

After the first progesterone peak (2,3 nmol/L) the animal showed no progesterone for more 45 days showing after that an oscillatory pattern that resembles a cycle, with a peak of 3,54 nmol/L. Lactation's stops seventy-four days after the first progesterone surge in the milk. In the cattle the first estrus after beginning of puberty or parturition is preceded by a short luteal phase, this is necessary for estrus manifestation¹³. In the tapir we realize the same situation, there was a small luteal phase that precedes the first full luteal production before the end of lactation. That may indicate a lower evolution stage when compared with horses that didn't show this hormonal pattern.

The curve of progesterone excretion didn't show a regular shape, but if we consider that progesterone concentration in the milk can vary in function of suckling interval, and the short storage ability (the udder is similarly to the mare). Correcting the curve shape according to expected, we obtain what is represented in Figure 1, where we can observe a 31-day cycle.

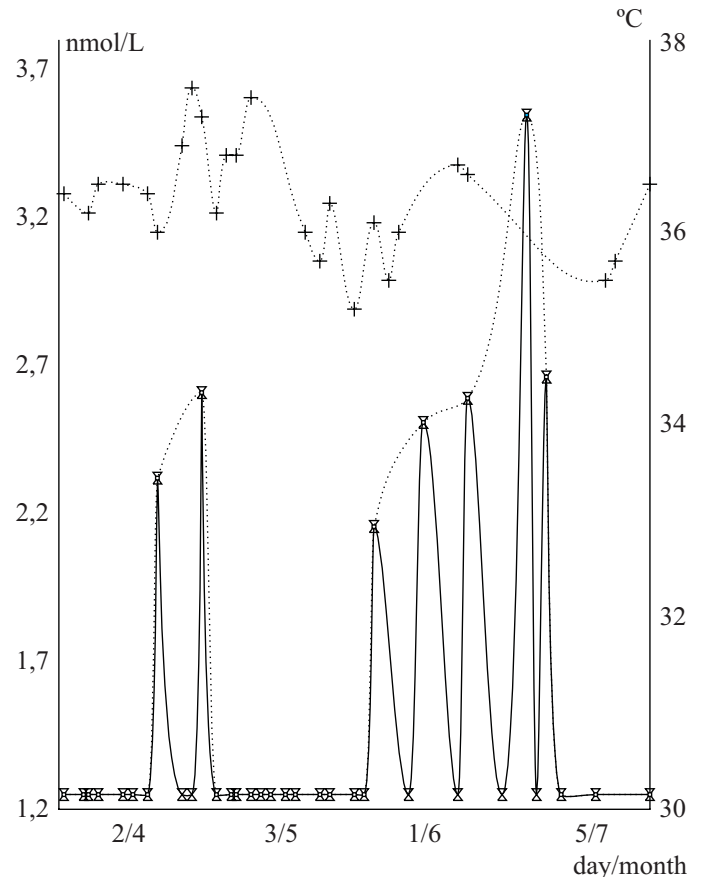


Figure 1

Fluctuation of milk progesterone (6 nmol/L) on female *Tapirus terrestris* observed in a postpartum period. The birth was on 11th November but the progesterone secretion starts at April. It can be observed a short cycle before a bigger, and the irregular progesterone excretion. The rectal temperature (+ °C) showed no correlation with the progesterone variation.

The beginning of progesterone production in April (Fall) may suggest the absence of a peripheral seasonal control. The seasonal breeding is such an extremely important mechanism for regulating the birth interval in mammals living in Arctic and temperate regions of the world. Animals who live in tropical areas are not supposed to have such mechanism¹⁴.

During the vaginal cytology collection, a large amount of fat secretion, produced locally by Bartolin glands like, was observed at the entrance of the vulva's lips. This fat secretion plus the perfect vulva labium's closure keeps the vaginal medium integrity even during the period that the animal is inside de water. Despite these anatomical features, we found in the vaginal smears a large amount of microorganisms (bacteria, coccus, diplococcus and bacillus). The vaginal epithelium cell type varied during the experimental period. Only in the day of the first progesterone peak there was a diminution of epithelial cells and an increase in the leukocyte's number. After this period there was a predomination of leukocytes' cells.

For twice we observed a calcium carbonate secretion from urinary via. This secretion first observed in the female occurred also in the male. The ejection of this ink-like secretion allowed the animal to colored the stall wall and trees of the enclosure. All

the obstacles by the animals high were virtually white. In the beginning of the milk collection we found in the ground of the enclosure a calcium carbonate bezoar and we never found another again. It is important to salient that the animals were feed with a horse concentrate, fruits and vegetables but no specific calcium extra source.

The rectal temperature oscillated without any relationship with the progesterone milk fluctuation.

From this we can conclude that in the studied animal we found a prolonged lactational anestrus (with nearly 5 months). Soon after the beginning of progesterone detectable milk levels the animal stops cycling, showing that during the lactation the tapir seldom cycle. The female started cycling in April (Fall)

showing no positive photoperiod dependence, if compared with equines. The progesterone milk quantification showed useful for assessing reproductive status in this specie. Like for horses the vaginal cytology cannot be used as reference for estrus cycle study. During tapir's lactation the rectal temperature showed no variation at the beginning of progesterone production.

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RESUMO

O tapir ou anta, descrito como o maior mamífero terrestre brasileiro, pertence à ordem Perissodactyla, subordem Hippomorfa, superfamília Tapiroidea e família Tapiridae. Na floresta úmida está envolvido com a dispersão de sementes em função das características do tubo digestivo. O acompanhamento do ciclo estral permite avaliar e compreender a atividade reprodutiva nas espécies animais. Nos animais silvestres, o estresse da contenção pode interferir na própria ciclicidade gonadal, uma das alternativas é o acompanhamento dos hormônios gonadais nas excretas. Buscamos neste trabalho o acompanhamento periódico da atividade gonadal através da quantificação de progesterona no leite. Utilizamos uma fêmea recém-parida no Zoológico de Araçatuba, da qual colhemos leite, esfregaço vaginal e temperatura retal. A progesterona foi quantificada por radioimunoensaio de fase sólida (Coat-a-Count, DPC®) com curva padrão fornecida pela F.A.O. O ensaio mostrou sensibilidade de 1,25 nmol/l com coeficiente de variação intra-ensaio de 15,36%. Durante a fase de lactação, a fêmea não apresentou níveis detectáveis de progesterona por 158 dias (de novembro a abril). O primeiro pico de produção foi de 2,3 nmol/l e apresentou duração de 5 dias. O segundo pico de 3,54 nmol/l teve uma duração de 23 dias. Setenta e quatro dias após o aparecimento da progesterona no leite a lactação cessou. Podemos concluir que a fêmea de tapir apresentou um período de anestro lactacional de aproximadamente 5 meses e voltou a ciclar no outono (abril) sugerindo pouca influência do fotoperíodo. A quantificação da progesterona no leite mostrou-se útil para o acompanhamento do ciclo estral na espécie, porém a variação da citologia vaginal e temperatura retal não apresentaram correlação com os níveis hormonais.

UNITERMOS: *Tapirus terrestris*; Leite; Progesterona; Reprodução Animal; Antas.

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