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Diurnal Rhythm of Plasma Oxytocin Concentration in Lactating Buffalo Cows

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Abstract: The aim of this study was analyze the diurnal rhythm of plasma oxytocin (OT) concentration in lactating buffalo cows during autumn and spring. Data were obtained from 10 females in May (autumn) and 10 females in September (spring) at 3-h intervals for 3 consecutive days. Cosinor and spectral analyses showed that, in autumn, 30-40% of the animals showed a significant 24-h rhythm of plasma OT concentration, whereas in spring the animals did not present a diurnal rhythm of plasma OT concentration. The majority of the acrophases for OT in autumn occurred at early morning and morning while in spring it was distributed along the day. Our study shows that diurnal rhythm of plasma OT concentration was more evident in some animals in the autumn that in the spring, what may suggest that photoperiod among others factors can influence in this response.

Keywords: hormone, bubalus bubalis, cosinor method, spectral analysis

Introduction

Biochemical, neuroendocrine, endocrine and behavior rhythms play an important role in many different aspects of the reproductive process in animals. A rhythmic release of hormones provides the endocrine system with a great deal of flexibility. The influence of photoperiod on the secretion of prolactin, growth hormone and gonadotropin has been described (Malpaux, 2006).

However, it is not known if diurnal variation influences oxytocin (OT) secretion. OT stored and released from the neurohypophyse is synthesized in magnocellular neurons whose cell bodies are located in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus. Oxytocin is also present in accessory magnocellular neurons of the preoptic area, the lateral hypothalamus, the anterior commissural nucleus, the nucleus circularis, the perifornical nucleus and near the third ventricle (Burbach et al., 2006).

OT is known to be primarily the mediator of milk ejection and uterine contractions during parturition. However, the hormone can also be involved in other reproductive process such as maternal, sexual and social behaviors, which include interactions with members of the same species (Uvnäs-moberg et al., 2001; Williams et al., 2001; Argiolas and Melis, 2004; Leng et al., 2008). In addition, oxytocin has been implicated as a neurotransmitter in the regulation of feeding and grooming (Lee et al., 2009), stress response, anxiety (Amico et al, 2004; Slattery and Neumann, 2010) and osmotic regulation (Antunes-Rodrigues et al , 2004).

Diurnal rhythm of plasma OT concentration has been reported in some mammalian species (Forsling, 2000; Devarajan and Rusak, 2004). Temporal changes in these secretory episodes are believed to account for the circadian pattern of circulating OT.

Despite the importance of OT in many physiological functions, is no report in the literature about the diurnal rhythms OT in buffalo. Thus, in the present study we aim to evaluate diurnal variations in plasma OT for this specie in two periods of the year (autumn and spring).

Materials and Methods

Data were collected at the Estação Experimental de Zootecnia Vale do Ribeira, at the Registro county in State of São Paulo, Brazil, latitude 24º43'S and longitude 47º53'W, at an altitude of approximately

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25 m. It was used a herd of river buffalo (Bubalus bubalis) predominantly Murrah phenotype, which was managed and selected for milk production. Cow´s age ranged from 3-10 years (1 to 5 months in lactation). The study was conducted in May (autumn, n=10) and September (spring, n=10), with a natural photoperiod of 11L:13 D and 12 L:12 D and temperature 17.3 and 19.5ºC respectively. Artificial light with intensity of approximately 30 lux was used to obtain the samples during the nocturnal period.

During the whole duration of the study, the animals were confined to a barn with free access to water, and fed with Bacharia sp twice a day, at 7:00 and 15:00h in May and 8:00 and 15:00h in September. Calves were allowed to suckle at 6:00 and 15:00h. Blood samples were collected from the jugular vein into a tube with heparin (Vacutainer®), at 3-hour intervals for 72h, and immediately placed on ice. Plasma was obtained by centrifugation at 1240g for 15 min and stored at –20ºC until hormonal assay.

Plasma was submitted to the process of OT extraction before radioimmunoassay (RIA). OT-specific antibody and standards were kindly provided by Wright State University, OH, USA. Nonspecific antibody against rabbit gamma-globulin was produced by the Laboratory of Neuroendocrinology (FMRP-USP). The iodinated hormone was purchased from Dupont Lab. The intra- and interassay coefficients of variation for the OT RIA were 4.5 and 8.3%, respectively. Standard curves generated with ^{125}IOT showed a sensitivity of 0.16 pg/tube and a linearity 0.12 and 15.6 pg/tube. Samples were assayed in duplicate.

The daily average of plasma P_4 concentration was analyzed to indicate the reproductive status of the animal. Plasma was submitted to the process of P_4 extraction before RIA. P_4-specific antibody and non-specific antibody against rabbit gamma-globulin were produced by the Laboratory of Neuroendocrinology (FMRP-USP). Standards were purchased from Sigma Chemical Comp., and tritiated hormone was from Amersham Pharmacia Biotech. The intra- and interassay coefficients of variation for P_4 RIA were 4.1 and 14.4%, respectively. Standard curves generated with [1,2,6,7^{3}H] P_4 showed sensitivity of 47.3 pg/tube and linearity of 31.3 and 2000 pg/tube. Samples were assayed in duplicate.

The Cosinor method was used for data analysis to determine the rhythmometric parameters for rhythm plasma OT concentration for analysis of fit, with a 24–h period, by the least squares method (Halberg et al., 1977). The rhythm parameters of each variable were determined using 00:00h as the acrophase reference (Nelson et al., 1979). The potency for temporal series plasma OT concentration was determined by spectral analysis by FFT technique (Fast Fourier Transform).

Results

During autumn, the MESOR values ranged from 4.15 to 6.44 pg/ml for plasma OT concentration (table 1). Amplitude (AMP) values varied from 0.49 to 1.45 pg/ml. The acrophases (ACRO) of plasma OT concentration could be divided into three groups, according to the time of the day: (1) early morning (04:30, 04:51, 05:02, 05:11, 05:37 and 05:55 h), (2) morning (07:53, 08:18 and 09:16 h) and (3) early afternoon (12:08 h). The percent rhythm (%R) obtained for plasma OT concentration was 3.35 to 27.25%.

Three animals presented a diurnal rhythm of plasma OT concentration. Spectral analysis showed statistically significant 24-h periods for plasma OT concentration in 4 of the 10 animals ($p<0.05$, table 2).

Figure 1 shows representative results, with presence (panels A and B) and absence (panel C) of diurnal rhythm of plasma OT concentration during autumn.

Among the animals showing OT diurnal rhythm, two presented daily average of plasma P_4 concentration <1.0 ng/ml and two >1.0 ng/ml. One of the females was pregnant, according to the registers of the farm. Among the animals that did not show OT diurnal rhythm, one presented plasma P_4 concentration >1.0 ng/ml, whereas for the others it was <1.0 ng/ml.

During spring, animals did not showed diurnal rhythm of plasma OT concentration (table 3). MESOR values for plasma OT concentration ranged from 4.01 to 5.26 pg/ml. AMP values varied from 0.21 to 1.04 pg/ml. The ACRO of plasma OT concentration could be divided into four groups, according to the time of the day: (1) early morning (01:13 and 02:22 h), (2) morning (08:05 h), (3) early afternoon (13:00 and 13:07 h) and night (18:46 to 23:39 h). Figure 2 shows absence of diurnal rhythm of plasma OT concentration (panels A and B) during spring.

During spring, daily average of plasma P_4 concentrations was <1.0 ng/ml in two animals and >1.0 ng/ml in eight, among which six were pregnant according the farm registers.

Discussion

To our knowledge, this is the first report that describes a 24-h rhythm of plasma OT concentration in lactating buffalo cows. In addition, we showed that the highest concentrations of OT (acrophases) occurred at early morning and morning during autumn, while in spring the acrophases were distributed throughout the day. Previous reports have described circadian rhythm in plasma OT concentration in rats (Devarajan and Rusak, 2004) and man (Forsling, 2000).

In lactating Rhesus monkeys (Macaca mulatta), variations in the concentration of cerebrospinal fluid OT were shown to be independent of the suckling stimulus and plasma OT concentration. Lactating animals also showed a normal circadian variation of cerebrospinal fluid OT concentration, with peak and nadir levels during light and dark hours, respectively (Amico et al., 1990).

The circadian activity is controlled by endogenous circadian clocks, of which the best understood is the influence of suprachiasmatic nuclei (SCN) (Goldman, 2001). Connections between the SCN and NPV have been demonstrated (Tousson and Meissl, 2004), therefore output from the SCN could affect the activity of magnocellular neurons and the rhythm in plasma OT concentration.

The fact that some of the animals in analyzed in the present study did not present diurnal rhythm of OT concentrations in the autumn suggests that the basis of the control of this rhythm is probably complex. This is expected, given the large number of transmitter systems, including amino acids, catecholamines, endogenous opioids, and neuropeptides that regulate hypothalamus neurohypophyse axis (Leng et al., 1999).

It has been shown that excitatory inputs to PVN and SON neurons originate mainly from distant brain areas (Csaki et al., 2000; Csaki et. al., 2002). Inhibitory inputs, in contrast, originate from cell populations that are located within, and in the vicinity of SON. GABAergic interneurons, for instance, can be found within the SON and in the perinuclear zone dorsal to it (Gies and Theodosis, 1994). The occurrence of rhythm may involve an integration of excitatory neurotransmitters or simply rhythmic secretion of a single excitatory neurotransmitter. However, the absence of rhythm may involve reduced excitatory neurotransmitters or increased secretion of inhibitory neurotransmitters.

In addition, desynchronization of the secretory patterns of two or more neurotransmitters could result in loss of the circadian OT rhythm. Uncoupling of the rhythms of these hypothalamic regulators could then explain why some of the animals did not present diurnal rhythm for this hormone.

Our results showed diurnal rhythm of plasma OT concentration in 40% of the buffalo cows studied in autumn, and no diurnal rhythmicity in spring. Theses results suggest that photoperiod can influence OT diurnal variation, as already reported for the secretion of other hormones such as prolactin, growth hormone, gonadotrophin releasing hormone and gonadotropin for other species (Malpaux, 2006).

Buffalo species shows a seasonality reproductive behavior that is not dependent on diet, food availability or metabolic status, but is mainly related to climate and photoperiod. Roy and Prakash (2007) observed in buffaloes during the summer, a circadian rhythmicity of the prolactin profile. In the literature there is no description of relationships about the diurnal rhythms OT in buffalo, as well as the influence of photoperiod in this response.

Among the animals showing OT diurnal rhythm in autumn, two presented daily average of plasma P_4 concentration < 1,0 ng/mL and two >1,0 ng/mL. Our results also showed that, in autumn, among the animals presenting diurnal rhythms in the plasma OT concentrations according to the plasma P_4 concentrations, one was pregnant, one was in the luteal phase and two were in the phase follicular or anestrus. Among the animals that did not show OT diurnal rhythm, one presented plasma P_4 concentration >1,0 ng/mL (phase luteal), whereas for the others it was < 1,0 ng/mL (follicular phase or anestrus).

During spring, daily average of plasma P_4 concentrations was < 1,0 ng/mL in two animals (phase follicular or anestrus) and >1,0 ng/mL in eight, among which six were pregnant according the farm registers and two were in the luteal phase.

Variation of OT secretion during the oestrous cycle has been described in the literature. In female rats, plasma oxytocin peaked in control animals during proestrus and. oxytocin content decreased in the paraventricular and supraoptic nuclei during proestrus and estrus compared to diestrus and increased in the neurohypophysis during proestrus morning (Caligioni and Franci, 2002).

In ewe, OT and P_4 showed the expected patterns of plasma concentrations, increasing during the early phase, reaching a plateau and declining either before

(OT) or at (P_4) luteolysis. OT values, however, did not vary at different times of the day, or show diurnal rhythm for OT in this phase (Whates et al., 1992). Basal values of plasma OT concentration are low during the 4 days around oestrus, reaching maximum values around day 8 (Whates et al., 1993).

In agreement with Forsling (2000) the diurnal pattern of neurohypophysial hormone secretion in affected by reproductive status in the female, probably as a result of the changing concentrations of ovarian steroids.
In the literature there is no description of relationships between the rhythmometric analyses of plasma OT concentration during estrous cycle in buffalo.

In conclusion, our study shows that diurnal rhythm of plasma OT concentration in buffalo was more evident in autumn than in spring, suggesting that photoperiod, among other factors, may influence this response. Further studies are needed to clarify the influence of circadian changes in OT concentration on the reproduction in buffalo.

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Table 1. Rhythmometric parameters of plasma OT concentration in lactating buffalo cows in autumn obtained by the Cosinor method.

Animals	MESOR	AMP	ACRO	%R	PVAL
1	4.20	1.26	05:55	27.25	0.003
2	5.41	0.61	05:02	4.26	0.457
3	4.21	0.49	05:11	3.35	0.542
4	4.15	0.57	05:37	8.63	0.197
5	4.16	0.84	07:53	14.01	0.077
6	4.91	0.85	09:16	13.98	0.067
7	4.87	1.24	04:30	22.46	0.010
8	6.44	1.45	08:18	14.83	0.050
9	4.68	0.59	04:51	5.70	0.338
10	4.27	0.90	12:08	9.26	0.166

MESOR is the mean for the cosine curve adjusted to the data, AMP is its amplitude, ACRO is the acrophase in hours, % R is the percent of rhythm and PVAL is the $p \leq 0.05$ probability that the amplitude for 24-h periodicity is zero. The units utilized for MESOR and AMP was pg/ml.

Table 2. Statistically significant periods ($p \leq 0.05$) of plasma OT concentration rhythms in lactating buffalo cows in autumn, reported in hours.

Animals	Oxytocin
1	24
5	24
7	24
8	24

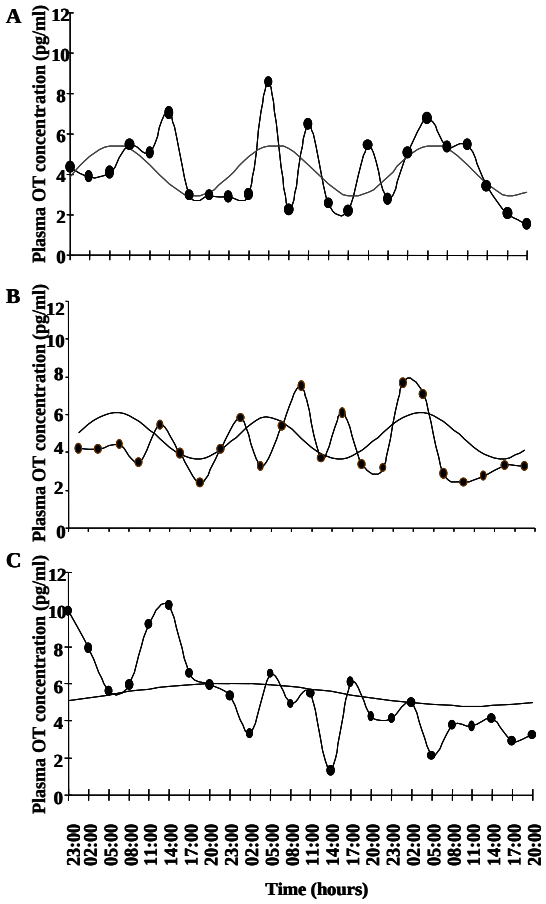

Figure. 1. Plasma OT concentrations measured at 3-h intervals for 72 consecutive hours for the buffalo cow 1 (Panel A; P < 0.003), 2 (Panel B; P< 0.01) and 2 (Panel C; P=0.530) in autumn. The dotted curve (--●--) represents plasma OT concentrations and the continuous curve (-----) the values obtained by the Cosinor method.

Table 3. Rhythmometric parameters of plasma OT concentration in lactating buffalo cows in spring obtained by the Cosinor method.

Animals	MESOR	AMP	ACRO	%R	PVAL
16	4.55	0.54	01:13	3.47	0.530
17	5.01	0.66	13:00	5.33	0.373
18	4.92	0.39	20:45	2.30	0.658
19	4.77	0.64	18:46	5.83	0.339
20	4.01	0.89	13:07	9.08	0.180
21	4.29	0.83	20:52	5.72	0.378
22	4.62	1.04	23:39	11.71	0.113
23	5.26	0.35	08:05	1.01	0.837
24	4.21	0.47	21:50	3.87	0.501
25	5.20	0.21	02:22	0.38	0.934

MESOR is the mean for the cosine curve adjusted to the data, AMP is its amplitude, ACRO is the acrophase in hours, % R is the percent of rhythm and PVAL is the $p \leq 0.05$ probability that the amplitude for 24-h periodicity is zero. The units utilized for MESOR and AMP was pg/ml.

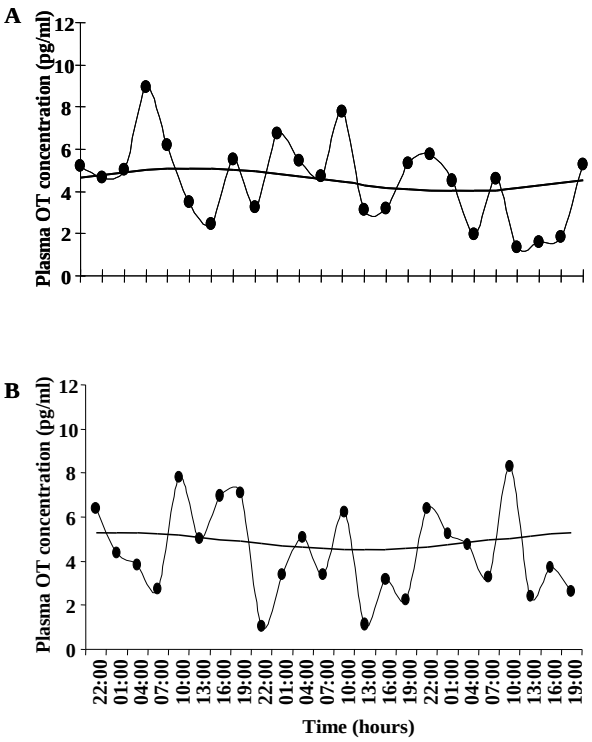

Figure 2. Plasma OT concentrations measured at 3-h intervals for 72 consecutive hours for the buffalo cow 16 (Panel A; P=0.530) and 18 (Panel B; P=0.658) in spring. The dotted curve (--●--) represents the plasma OT concentrations and the continuous curve (-----) the values obtained by the Cosinor method.