



## Research

# Monitoring of total locomotor activity in mares during the prepartum and postpartum period



Claudia Giannetto<sup>a</sup>, Marilena Bazzano<sup>a</sup>, Simona Marafioti<sup>a</sup>, Cristiano Bertolucci<sup>b</sup>, Giuseppe Piccione<sup>a,\*</sup>

<sup>a</sup> Department of Veterinary Sciences, University of Messina, Messina, Italy

<sup>b</sup> Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy

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## ABSTRACT

The aim of this study was to investigate the daily rhythms of total locomotor activity (TLA) in mares during the prepartum and postpartum periods and the existence of these rhythms at the time of birth in neonatal foals. For this purpose, TLA was recorded in 9 mares starting from the third day preceding the date of foaling until 3 days after foaling. The TLA was recorded in the newborn foals starting from 2 to 6 hours after birth until the third day of life. Animals were housed in individual straw-bedded boxes (4.0 × 3.5 m) under natural photoperiod and ambient temperature. Animals were fed twice a day (7 AM; 5 PM) and were allowed to go to the paddock during the day (from 10 AM to 4 PM). Mares showed a diurnal activity, and the total amount of daily activity statistically changed during the 6 days of monitoring. Activity was statistically higher in the prepartum period than in the postpartum period. In foals, a statistical increase of the total amount of daily activity was observed in the 3 days of monitoring, but no differences were observed in the amount of activity recorded during the photophase and the scotophase. Both in mares and foals, rhythmicity was observed on the second and second days postpartum, and the acrophase of rhythm did not change during the monitored period. The mean level and amplitude of rhythm were higher in foals than in mares. In mares, the amplitude was lower during day 3 postpartum compared to days 1 and 2 prepartum. Robustness of rhythm in mares was lower in day 3 postpartum compared to days 1 and 3 prepartum. In conclusion, although TLA significantly changed from the 3 days prepartum to the 3 days postpartum, the acrophase of rhythm was the same in all experimental conditions and was opposite to that observed in foals.

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## Introduction

Biological rhythms allow animals to exploit the opportunities offered by the periodic nature of the environment (Pittendrigh, 1993). They have been observed at all levels of organization, from behavior to gene and protein expression (Brown and Schibler, 1999; Gachon et al., 2004; Piccione et al., 2014). Daily oscillations have been recorded under natural and controlled conditions in several animal species for a variety of physiological and behavioral parameters, such as locomotor activity, eating and drinking, excretion,

learning capability, heart rate, blood pressure, body temperature, and hormone secretion (Refinetti, 2006). It is well known that locomotor activities exhibit daily rhythmicities. The daily regime of different equine species has been widely studied since the 1960s. Gill (1991) provided one of the first long-term registrations of behavioral activities in horses. He showed a daily rhythm of motor activity in horses under several conditions, with different patterns of activity from day to day because of weather, temperature, grazing, and abundance of flies. Studies conducted on Przewalski horses maintained under semireserve, on free-living Camargue foals, and domestically managed Dutch Warmblood foals showed that behavioral activities occurred mainly during the photophase (Boy and Duncan, 1979; Berger et al., 1999; Kurvers et al., 2006; Houpt et al., 2001). Grazing behavior is the main daily activity of the horse and it consists of feeding as well as locomotor activity. The

\* Address for reprint requests and correspondence: Giuseppe Piccione, Department of Veterinary Sciences, University of Messina, Polo Universitario dell'Annunziata, Messina 98168, Italy, Tel: +39 090 3503584; Fax: +39 090 3503975.

E-mail address: [giuseppe.piccione@unime.it](mailto:giuseppe.piccione@unime.it) (G. Piccione).

time spent grazing depends on the structure and dispersion of the patch on which animals prefer to feed, on the season, on the age, on the sex, and on the forage availability (Haupt et al., 2001). In horses housed in stalls with or without paddocks, locomotor activity showed a diurnal pattern and its acrophase always occurred in the middle of the photoperiod (Piccione et al., 2008a).

In several species of mammals, age-related changes in the circadian timing system have been well documented. In foals, the fetal maturation that makes the passage to extrauterine life begins during the last 5 days of pregnancy and ends within the first 7–10 days of life (Le Blanc, 1996). Furthermore, the maturation of the central pacemaker and peripheral oscillators was not simultaneous after birth (Piccione and Giannetto, 2011a). In foals, the daily rhythm of body temperature emerges within the first 10 days of life and matures fully during the first month after birth (Piccione et al., 2002). Different from that observed for the body temperature, blood pressure did not show a daily rhythmicity in foals monitored for 40 days after birth (Piccione et al., 2008b). The research on ontogenic changes of circadian rhythm mirrors the developmental progress in chronobiological research. Early investigations were aimed at evaluating the endogenous, hereditary nature of circadian rhythms (Weinert, 2005). On the basis of this knowledge, the aim of this study was to investigate (1) the existence of daily rhythm of locomotor activity at the time of birth in neonatal foals and (2) the changes of locomotor activity in mares during the 3 days prepartum and the 3 days postpartum.

## Materials and method

### Animals

The study was carried out in Sicily, Italy (latitude 37.46°N; longitude 14.93°E). The length of photoperiod was about 12 hours and was calculated using a day length calculator algorithm provided by the US National Oceanic and Atmospheric Administration. The mean minimum ambient temperature was 10°C and mean maximum ambient temperature was 16°C.

We recorded total locomotor activity (TLA) in 15 mares housed at the same breeding center, starting from the week preceding the expected date of foaling (prepartum) until 3 days after foaling (postpartum) with the informed owner consent. Nine mares of different breeds and ages (Table 1) that delivered in the same week at midnight  $\pm$  1 hour and their foals were enrolled in the study. The foals were monitored starting from 4  $\pm$  2 hours after birth until the third day of life. The animals were housed in individual straw-bedded boxes (4.0  $\times$  3.5 m) at the same breeding center and were kept under natural photoperiod and ambient temperature. No artificial lights were turned on during the night. The animals were fed twice a day (7 AM; 5 PM), and water was available ad libitum. The diet consisted of 6  $\pm$  1 kg/day hay and 5  $\pm$  0.5 kg/day concentrates

(crude protein, 16%; crude fat, 6%; crude fiber, 7.35%; ash, 10.09%; sodium, 0.46%; lysine, 0.85%; methionine, 0.35%; and omega-3, 0.65%). Animals were allowed to go to the paddock during the day (from 10 AM to 4 PM). The mares had no social interaction with the other mares in the month before expected partum. After parturition, the newborns had no contact with other mothers and foals during the 3 days of monitoring. General animal care was carried out by professional staff not associated with the research team only during the daytime. All treatments and housing and animal care procedures were carried out in accordance with the standards recommended by the European Union Directive 2010/63/EU for animal experiments.

### Locomotor activity

TLA pattern was recorded using the Actiwatch Mini (Cambridge Neurotechnology Ltd, Cambridge, UK) actigraphy-based data logger that records a digitally integrated measure of motor activity. The device was attached to the mane near the withers both in mares and foals that were accepted by the horses without any obvious disturbance. This activity acquisition system is based on miniaturized accelerometer technologies and it has been previously used to record locomotor activity in horses (Bertolucci et al., 2008). The total activity recorded was the result of all movements, which includes different behaviors such as feeding, drinking, walking, grooming, and small movements during sleep, independent from the animal's position, as lying or standing.

Activity was monitored with a sampling interval of 5 minutes. The total amount of daily activity was calculated as the arithmetic mean of the 288 data points recorded during 24 hours. The amount of activity during photophase or scotophase was calculated using Actiwatch Activity Analysis 5.06 (Cambridge Neurotechnology Ltd, Cambridge, UK). Actiwatch Activity Analysis 5.06 was also used to draw actograms, a type of graph commonly used in circadian research to plot activity against time.

### Statistical analysis

All results were expressed as mean  $\pm$  SD. Data were normally distributed (Kolmogorov–Smirnov test). One-way for repeated measures analysis of variance (ANOVA) was applied on the total amount of daily activity to compare the 6 days of monitoring in mares (prepartum and postpartum) and the 3 days of monitoring in foals. Two-way for repeated measures ANOVA was applied to establish if mares and foals were mainly diurnal or nocturnal and to compare the amount of activity during the photophase and the amount of activity during the scotophase between the 6 days of monitoring in mares (prepartum and postpartum) and the 3 days of monitoring in foals.

In addition, a trigonometric statistical model was applied to the average values of each time series, as to describe the periodic phenomenon analytically, by characterizing the main rhythmic parameters according to the single cosinor procedure (Nelson et al., 1979). Four rhythmic parameters were determined: mean level, amplitude (the difference between the peak, or trough, and the mean value of a wave), acrophase (the time at which the peak of a rhythm occurs), and robustness (strength of rhythmicity). One-way for repeated measure ANOVA was also applied to evaluate an effect of prepartum and postpartum period on the rhythmic parameters observed during the 6 days of monitoring in mares. A paired Student *t* test was applied between rhythmic parameters observed in days 2 and 3 in foals. Statistical analysis was performed using the Prism package (GraphPad Software Inc., San Diego, CA); 2alpha = 0.05 was considered statistically significant. The Bonferroni multiple comparison test was applied for post hoc comparison.

**Table 1**  
Breed, age (years), gestation length (days), and parity (+, multiparous; –, primiparous) of experimental mares

Breed	Age	Gestation length	Parity	Time of delivery
Selle Français	17	337	+	11 PM
Standardbred	4	360	–	12:35 AM
Italian Saddle	16	341	+	12:15 AM
Paint	8	350	+	1 AM
Italian Saddle	9	333	+	12:45 AM
Italian Saddle	3	345	–	11:20 PM
KWPN	17	328	+	11:50 PM
KWPN	10	331	+	11:30 PM
Italian Saddle	12	339	–	12:10 AM

KWPN = Koninklijk Warmbloed Paardenstamboek Nederland.

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