Acta Veterinaria (Beograd), Vol. 58, No. 1, 25-31, 2008.

DOI: 10.2298/AVB0801025C UDK 619:613.1:636.1

THE INFLUENCE OF SEASON AND AGE ON CIRCULATING MELATONIN AND LEPTIN CONCENTRATIONS IN LIPIZZAN FILLIES

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(Received 1. May 2007)

Daily serum melatonin and leptin concentrations in 48 Lipizzan fillies, aged 1 to 3 years, have been measured during one year. Mean melatonin concentrations ranged from 7.08 ± 0.43 pg/mL in 3-year olds to 9.02 ± 0.73 pg/mL in yearlings (P>0.05). In all age groups, circulating melatonin concentrations peaked in spring and early autumn and decreased in summer and early winter (P<0.001). The decrease of circulating melatonin levels could serve as a signal for resumption of ovarian activity in spring and for onset of anoestrus in autumn, but further investigations are needed to confirm this hypothesis. Mean annual leptin concentrations ranged from 2.55 ± 0.15 ng/mL in yearlings to 3.94 ± 0.32 ng/mL in 3-year olds (P<0.01); age-dependent differences were also observed during monthly samplings. In all age groups, the circulating leptin values were lowest in early spring and peaked in autumn (P<0.001). The rise of leptin levels in spring coincides with the period of resumption of ovarian activity and the decrease in winter with the anoestrous period of Lipizzan fillies, thus supporting the findings about the role of leptin in the regulation of the seasonal reproductive activity in mares.

Key words: horses, seasonal biorhythm, leptin, melatonin

INTRODUCTION

Mares are seasonal breeders, manifesting cyclic ovarian activity from early spring to autumn and anoestrus during the winter. Some non-pregnant mares remain cyclic throughout the year (Adams and Bosu, 1988; Fitzgerald *et al.*, 2002; Brown-Douglas *et al*., 2004). The most important factor regulating the circannual endogenous rhythm of seasonal reproductive activity in animals, living in moderate latitudes is the duration of daylight. The role of environmental temperature and nutrition, which influence the reproductive processes by energy metabolism, have also been confirmed (Adams and Bosu, 1988, Nagy *et al.,* 2000).

The influence of daylight on the reproduction of animals is mediated by the epiphyseal hormone melatonin (Gerlach and Aurich, 2000; Santiago-Moreno *et*

al., 2005; Sliwowska *et al*., 2004). Its secretion follows a circadian pattern, being inversely proportional to the duration of daylight. The highest concentrations are thus measured during the night and the lowest during the day (Gerlach and Aurich, 2000). Melatonin secretion is highest in the period of short days during the autumn and winter seasons and lowest in the period of long days during spring and summer. Increased melatonin secretion in the autumn months inhibits secretion of hypothalamic gonadoliberins, followed by decreased pituitary secretion of gonadotropic hormones, which leads to winter anoestrus in the majority of mares. Decreased melatonin secretion in spring enables the activation of the hypothalamo-hypophyseal axis and resumption of ovarian activity (Gerlach and Aurich, 2000).

An important factor regulating animal reproduction is the hormone leptin, secreted by cells of the white adipose tissue, in a manner directly proportional to the amount of body fats. Leptin regulates gonadotropin secretion by influencing the hypothalamo-hypophyseal axis. Direct leptin action on ovaries, uterus and testes was also confirmed (Houseknecht *et al.,* 1998; Chilliard *et al*., 2001; Spicer, 2001). The role of leptin in reproduction is confirmed by decreased gonadotropin secretion in females expressing negative energy balance, which leads to delayed puberty and cyclic ovarian activity (Houseknecht *et al.,* 1998; Spicer, 2001; Henry *et al.,* 2001; Adam *et al*., 2003; Barb and Kraelin, 2004). On the other hand, cycling ovarian activity is present in non-pregnant mares with good body condition during winter months (Fitzgerald *et al.*, 2002). Irrespective of energy status, circulating leptin values are increased during pregnancy, are higher in males than in females and in older animals than in younger ones (Houseknecht *et al.,* 1998, Chilliard *et al*., 2001, Spicer, 2001).

Despite very thorough studies of circadian melatonin fluctuations in different seasons (Gerlach and Aurich, 2000; Diekman *et al.,* 2002; McMannus and Fitzgerald, 2003; Sliwowska *et al*., 2004; Santiago-Moreno *et al*., 2005), no data is available on seasonal fluctuations of this hormone during the daylight hours. The aim of our work was to study seasonal fluctuations of daily circulating melatonin values in yearlings, two and three-year old Lipizzan fillies with respect to the time of year and age of animals. Additionally, we measured circulating leptin values to discover any correlations with the seasons of the year and the age of the animals.

MATERIAL AND METHODS:

The study was performed on 48 spring-born Lipizzan fillies (15 yearlings, 16 two-year olds and 17 three-year olds) from the Lipica stud farm. They were kept together, free in the stable, with outdoor paddocks, and fed twice a day with hay and oats. Drinking water was given *ad libitum*. The animals were kept in good body condition during the whole study.

Blood for hormone determination was collected monthly (around the 20th day of each month) for 12 consecutive months, starting with January, always between 10 and 12 a.m. These sampling hours were chosen to avoid any possible influence of nocturnal melatonin peaks. Blood samples were taken from the jugular vein using double-ended needles and evacuated tubes without

anticoagulant (Vaccuette, Greiner Labortechnic, Germany). Serum was kept frozen below -20°C until analyzed.

Serum leptin and melatonin concentrations were measured by commercial radioimmunoassay kits (Multi-Species Leptin RIA Kit, Linco, USA) and Melatonin direct RIA (Serum/Plasma), Immuno Biological Laboratories Hamburg (Germany). Intra-assay and inter-assay coefficients of variability (CV) for leptin were 11.3 % and 10.3 % respectively for high values (M = 28.16 ng/mL) and 13.2 % and 8.3 % respectively for low values (M = 4.35 ng/mL). Intra-assay and inter-assay CVs for melatonin were 8.94 % and 12.96 % respectively for high values (M = 71.19 pg/mL) and 10.27 % and 12.31 % respectively for low values (M = 4.21 pg/mL).

Statistical calculations were performed using *Statistical Package For Social Sciences for Windows, (SPSS) for Windows, Release 12.0* with subprograms General Linear Model, Paired Samples T-Test and Analyzes of Variance. Results were evaluated as statistically significant at the level of $P<0.05$, and presented as mean ± standard error (M±SE).

RESULTS

Mean whole-year daily melatonin concentrations were 9.02 pg/mL ± 0.73 pg/mL in yearlings, 8.32 pg/mL ± 0.66 pg/mL in two-year olds and 7.08 pg/mL ± 0.43 pg/mL in three-year olds; differences between the age groups were not significant, as was determined also for daily values each month. Mean daily melatonin concentrations in all examined age groups during the year are displayed in Figure 1. The differences between the highest values measured in

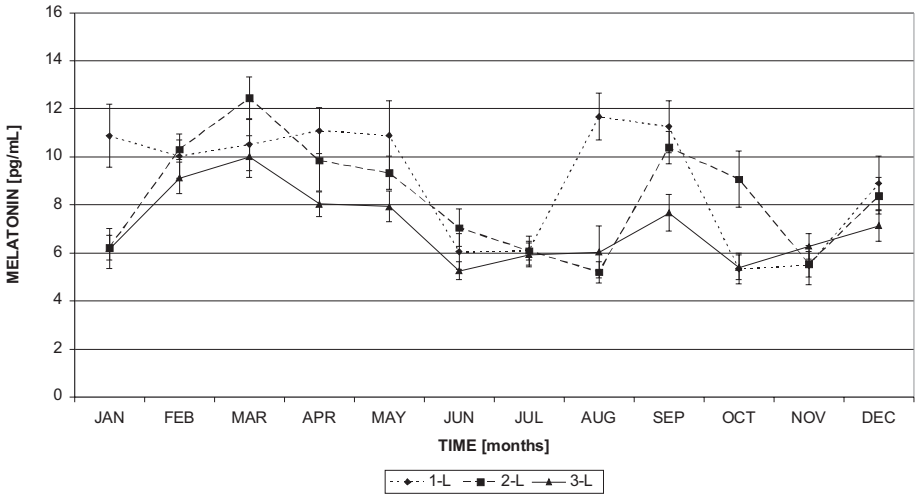

Figure 1. Daily melatonin concentrations (M±SE.) in Lipizzan fillies during the year (1-L – yearlings; 2-L – two-year olds; 3-L – three-year olds)

spring and autumn and the lowest in summer were significant ($P<0.001$) for all age groups.

Mean whole-year leptin concentration in yearlings was 2.55 ng/mL ± 0.15 ng/mL, in two-year olds 2.63 ng/mL ± 0.16 ng/mL and, in three-year olds, 3.94 ng/mL ± 0.32 ng/mL ($P<0.01$). The highest daily leptin values (Figure 2) in three-year olds and the lowest in yearlings were also present at monthly samplings ($P<0.01$). The lowest circulating leptin concentration was found from January to March and the highest from August to December ($P<0.001$) in all age groups (Figure 2).

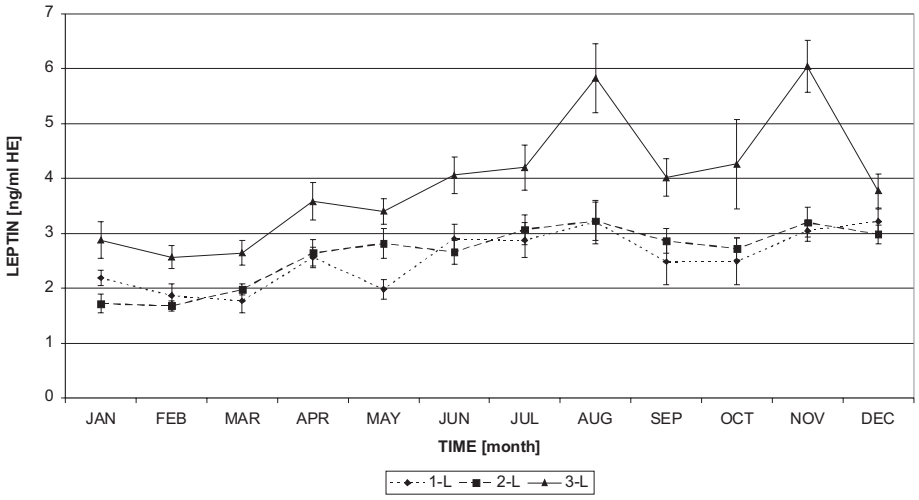

Figure 2. Daily leptin concentrations (M ± SE) in Lipizzan fillies during the year (1-L – yearlings; 2-L – two-year olds; 3-L – three-year olds)

DISCUSSION

Circulating melatonin concentrations in animals fluctuate following a circadian pattern with elevated values at night and low values during the day. Thus, the amount of secreted melatonin in animals, living in moderate latitudes, is higher during winter than during summer (Gerlach and Aurich, 2000; Sliwowska *et al*., 2004; Santiago-Moreno *et al*., 2005). Melatonin concentrations in the studied Lipizzan fillies, measured before noon, were lower than in Quarter horse mares (Diekman *et al.,* 2002), which could be attributed to breed differences but also to the use of different methods for melatonin measurement. Age-dependent variations in melatonin concentrations were not observed in the Lipizzan fillies, and are therefore most probably not the reason for levels lower than in Quarter mares.

In Quarter mares (Diekman *et al.,* 2002), the differences between the daily and nightly concentrations were less pronounced than in other animal species and the lowest daily melatonin concentrations were determined in spring and the highest in winter. In Lipizzan fillies, the opposite situation was the case, with the highest values during the spring and lowest during the autumn, indicating a two-phase annual pattern in daily melatonin concentrations. The spring decrease of circulating melatonin values coincided with the resumption of ovarian activity, and the autumn decrease with the termination of ovarian activity (Čebulj-Kadunc *et al.,* 2006). Therefore, the fluctuations of daily melatonin concentrations could be a signal for the onset and the end of the seasonal reproductive activity in fillies; further investigations are needed to confirm this possibility.

Mean leptin concentrations were in the range set for horses (Fitzgerald *et al.*, 2002; Gentry *et al*., 2002; McMannus and Fitzgerald, 2003; Ferreira-Dias *et al*., 2005). The highest mean yearly and monthly leptin concentrations were measured in the oldest group of fillies (three-year olds). An age-dependent decrease of leptin concentrations has also been reported in other warm-blooded horse breeds and is most probably induced by the decreased amounts of adipose tissue (Fitzgerald *et al.*, 2002; Gentry *et al*., 2002; McMannus and Fitzgerald, 2003; Ferreira-Dias *et al*., 2005).

The lowest leptin concentration in fillies was determined in February or March, then it gradually increased to reach the highest values in September and remained at the high level until the end of the year. In 3-year olds leptin concentrations were significantly higher than in the other two groups and the fluctuations were more pronounced. High levels during the autumn and winter months most probably reflect good body condition. The slight autumn decrease is most probably not caused by decreased energy reserves of the organism. It could be attributed to the influences of the external factors, among them most probably lower environment temperature (Fitzgerald and McMannus, 2000). Increase of leptin concentration in spring coincides with the onset of seasonal reproductive activity and the decrease in autumn with its termination (Čebulj-Kadunc *et al.,* 2006). This confirms the findings of several authors about the role of leptin in seasonal reproductive activity in mares (Fitzgerald *et al.*, 2002; Gentry *et al*., 2002; McMannus and Fitzgerald, 2003; Ferreira-Dias *et al*., 2005).

ACKNOWLEDGEMENTS:
The research was supported by the Ministry of Science and Technology of Republic Slovenia. We thank Mr. Anton Pečovnik, dr. vet. med. from Lipizza stud farm, as well as Mr. Boštjan Drolc for his technical assistance. The authors also thank Professor Roger H. Pain for his English corrections.

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UTICAJ GODIŠNJEG DOBA I UZRASTA NA NIVO MELATONINA I LEPTINA U KRVI MLADIH KOBILA RASE LIPICANER

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SADRŽAJ

Dnevne koncentracije melatonina i leptina u serumu 48 konja pasmine Lipicanac starih 1-3 godine bile su merene preko godine. Prosečna koncentracija melatonina varirala je od 7,08 ± 0,43 pg/ml kod životinja starih tri godine do 9,02±0,73 pg/m kod godišnjaka ($P>0,05$). U svim starosnim grupama koncentracije melatonina u krvi dostigle su najveću koncentraciju u proleće i ranu jesen, a najmanju tokom leta i u ranu zimu ($P<0,001$). Smanjene vrednosti melatonina mogu služiti kao signal za ponovnu aktivaciju ovarijalne aktivnosti u proleće i za pojavu anestrusa u jesen, ali bi bile potrebne dalje pretrage za potvrdu ove hipoteze. Prosečne godišnje koncentracije leptina varirale su od 2,55 ± 0,15 ng/ml kod godišnjaka do 3,94 ± 0,32 ng/ml kod 3 godine starih konja ($P<0,01$). Očite su bile i razlike mesečnih vrednosti kod konja različite starosti. U svim starosnim grupama vrednosti leptina bile su niže u rano proleće, a dostigle su vrh u jesen ($P<0,001$). Porast vrednosti leptina u proleće koincidira sa pojavom ponovne ovarijalne aktivnosti, a pad u toku zime sa pojavom anestrusa kod mladih konja lipicanaca. Rezultati su u saglasnosti sa opštim saznanjima o ulozi leptina u regulaciji sezonske reproduktivne aktivnosti kobila.