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Determination Melatonin in Serum of Arabian Horses by HPLC in Summer and Winter Solstices in Ahvaz

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Abstract: The objective of this study was to evaluate changes in serum concentration of melatonin of Arabian horses in solstices of year in Ahvaz region. Blood samples from jugular vein of 80 Arabian horses were collected in winter and summer solstices. Serum was harvested from these blood samples by centrifugation. After preparation and derivation of serums for HPLC assay, samples were injected to HPLC column and melatonin was measured by area under curve based on standard curve of melatonin. For statistical analysis horses have been separated in age groups of less than 2 year, 2-5 year, 5-10 year and more than 10 years old with regards to sex type in each season. The results shows: 1) a negative relation in mean serum melatonin concentration between group of horses with less than 2 years old in summer season and total mares group in winter season (P=0.034). 2) a negative relation in mean serum melatonin concentration between age groups of less than 2 years old and group 5-10 years old in summer season (P=0.01). 3) There was no significantly deference between results in other groups (P>0.05). Present study shows the season did not significantly affect serum melatonin level in Arabian horses in Ahvaz region.

Key words: Arabian horse · Seasonal changes · Melatonin · HPLC · Ahvaz region,

INTRODUCTION

Seasonal changes have effects on reproduction in some animals [1]. The pineal gland is able to receive photo information and regulate the secretion of melatonin [2, 3]. Melatonin is a key player in controlling reproduction and circadian rhythm [4- 6]. In northern hemisphere, animals are exposed to more melatonin during the night and that during the longer periods of darkness in winter. Conversely, melatonin levels decrease during long days in summer [5, 7]. The localization of melatonin receptors in the hypothalamus and pituitary indicates a possible interaction of melatonin in regulation of reproduction in horse [8]. Even though 10 to 20% of mares tend to exhibit estrous cycles through the year, the horse is a seasonal polyestrous species with onset of the breeding season occurring in spring, associated with increase in daylight, temperature and availability of food [9]. During the fall, the duration of secretion of melatonin from the pineal gland is increased, reflecting the increased duration of darkness in horse. These longer nights may provide a cue for the mare to enter into anestrus [7]. Under natural lighting conditions, serum concentrations of melatonin are higher in anestrus mares during the breeding season 10].The short day length characteristic of fall and winter causes an increase in the daily duration of melatonin secretion and this result in decreased secretion of gonadotropic hormones and the cessation of ovulatory activity [11].

Exogenous melatonin administration is reported to decrease plasma testosterone concentrations in stallions and change the annual reproductive rhythm in pony mares [12]. In contrast, artificial photostimulation produced a sharp rise in testosterone levels in stallion [13].

Limited information is available about the seasonality of reproductive activity of the Arabian horse bred in Iran, especially related to melatonin. The aim of the present study was to determine melatonin concentrations in Arabian horses in solstices of year in Ahvaz region.

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MATERIALS AND METHODS

Animals: A group of 80 healthy Arabian mares and stallions, ranged from 7 months to 18 years of age were used. Mares were classified into four groups as follows: <2years, 2-5 years, 5-10 years and >10years. The horses were fed with the standard horse diet and housed in the stable and kept separately. The experiment was conducted under natural photoperiod.

Blood Collection: Peripheral blood samples (10 ml) were collected via jugular venipuncture from each horse into tubes (in morning) during winter and summer solstices in Ahvaz area of Iran. Sera were decanted immediately into eppendorfs after centrifugation at 3000 rpm for 10 minutes and stored at - 20°C until melatonin measurement.

Melatonin Measurement: Serum concentration of melatonin was measured by high-performance liquid chromatography (HPLC) according to modified protocols were available for determination of melatonin in serum by HPLC [14-17]. Serum was extracted with trichloroacetic acid. After centrifugation, the supernatant was evaporated and the residue was stored at -20°C until analysis by HPLC. The recovery of the extraction procedure was 81 ± 5.1% (mean ± SEM, n = 4). The dried residue was redissolved in 500 µl of the HPLC mobile phase consisting of 60/40 methanol/water (v/v) and then filtered through a 0.45 µm filter. The filtrate (20 µl) was applied to a chromatographic system equipped with a column (C18) and a UV detector (Shimadzu, Japan). The detector was operated at a wavelength of 254 nm. All separations were carried out isocratically at a flow rate of 0.6 ml/min using the above-mentioned HPLC mobile phase at 37°C. The melatonin concentration of samples was calculated based on area under curve (AUC) and equation of standard curve which achieved by serial dilution of melatonin (Sigma, USA).

Statistical Analyses: Two - way ANOVA was performed in order to examine the effects of gender or season differences on melatonin levels. We considered that gender ($p<0.01$) and season ($p<0.001$) significantly affected on melatonin levels. The statistical analyses were performed using a software program (SPSS, version 11).

RESULTS

Seasonal changes of mean serum melatonin concentrations in stallions and mares are presented in Figs. 1-4. The mean (± Standard Error) serum concentrations of melatonin in summer were 32.99 ±4.4, 45.9 ±7.4, 48.66 ±3.4 and 38.29 ± 5.1 pg/ml in 4 groups of horses respectively (Fig. 1). This concentration in winter was 41.8 ±4.9, 51.91±11.1, 47.28±9.2 and 43.4±9.7 pg/ml in 4 groups of horses respectively (Fig. 2). The serum melatonin concentration of stallions was 40.2±3.1 pg/ml in summer and 39.4 ± 6.1pg/ml in winter, while this amount in mares was 42.1± 4.8pg/ml in summer and 51.7±5.8 pg/ml in winter (Fig. 3). The total mean of melatonin in mares was higher than male (Fig. 4).

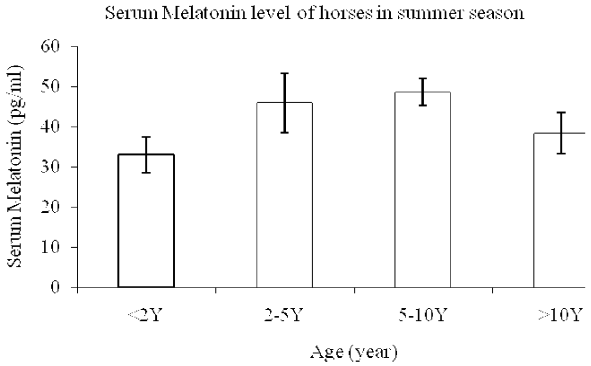

Fig. 1: Mean ± SE of serum melatonin level of horses in summer solstice

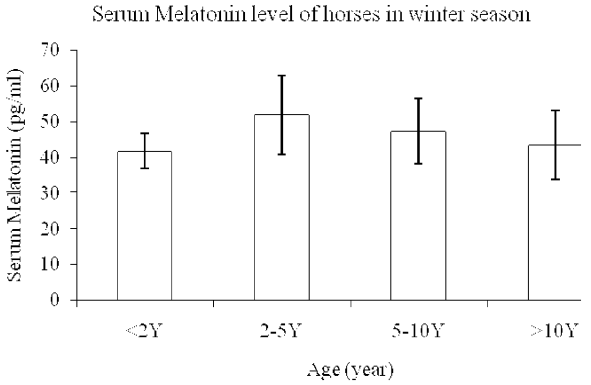

Fig. 2: Mean ± SE of serum melatonin level of horses in winter solstice

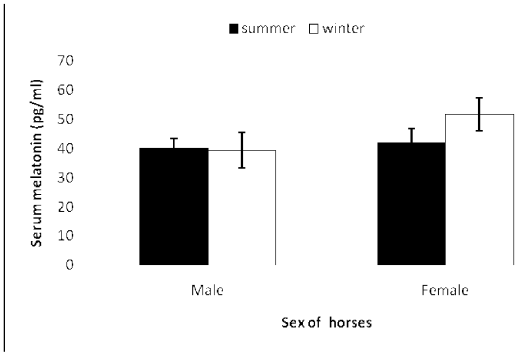

Fig. 3: Mean ± SE of serum melatonin level of male female horses in solstices.

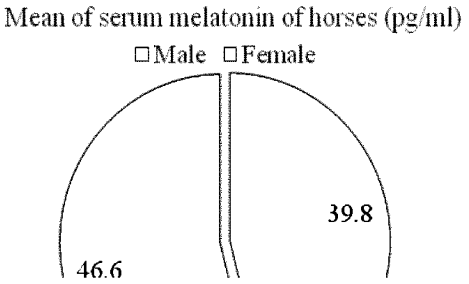

Fig. 4: Mean ± SE of serum melatonin level of male female horses.

The results shows: 1) a negative relation in mean serum melatonin concentration between group of horses with less than 2 years old in summer season and total mares group in winter season (P=0.034). 2) a negative relation in mean serum melatonin concentration between age groups of less than 2 years old and group 5-10 years old in summer season (P=0.01). 3) There was no significantly deference between results in other groups (P>0.05).

DISCUSSION

Photoperiodism has been known as a visual factor in regulating reproduction in seasonal breeders such as sheep, horses, hamsters, ferrets, deer, mink, skunks, voles and wallabies [10]. Regulation of reproductive activity via photoperiodic cues mediated by the secretion of melatonin from the pineal gland is reported in ewes [18, 19] but the relationship is less clear in mares [10, 20].

Under natural photoperiodic conditions, serum concentrations of melatonin are higher in anestrus mares [10, 21]. In response to artificial photoperiod, alterations in ovulatory season also exhibit in mares [22, 23]. In contrast to the human, the horse appears to possess a circadian pacemaker that is more amenable to rapid adjustment to a new photoperiod, suggesting in turn that their performance capacity at a new destination might be less compromised than in human athletes [24]. The annual change in photoperiod is considered the primary environmental factor that synchronizes seasonal reproductive activity in mares [11]. The onset of anestrus, body condition or fatness and a hormonal product of fat cells, leptin, may play a role in modifying the response to melatonin and photoperiod in the mare. Constant administration of melatonin modified the secretion of prolactin; specifically, treatment suppressed the increase in prolactin during spring months [25].

Information on photoperiod and the physiological reproductive season in Arabian horse is very limited. Ahvaz area has nearly two seasons that summer is longer than winter. The photoperiod difference between solstices at this area is about 6 hours. This status may be affect rate of melatonin [26]. We found that serum melatonin levels were greater winter solstice than summer solstice in mares (Fig. 3). Diekman *et al* reported that mean melatonin levels in mares were 16 pg/ml in June and 19.5 pg/ml in December. Guillaume *et al.* reported that mean concentration of nocturnal melatonin were 24 pg/ml during autumn in 110 pony mares [10]. Altinsaat *et al*. (2009) found similar changes in melatonin Arabian horses [27].

In conclusion, our observations suggested a role for melatonin in the breeding activity in the Arabian mare. However, additional study will be required to determine melatonin and other factors such as aging, nutrition and climate are involved in seasonal changes of in reproductive activity of Arabian horses in Ahvaz area.

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