Determination Melatonin in Serum of Kurdish Horses by HPLC in Kermanshah Region at Breeding Season

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Abstract: The objective of this study was to evaluate changes in serum concentration of melatonin of Kurdish horses Kermanshah region at breeding season (February to June of 2010). Blood samples from jugular vein of 40 Kurdish horses were collected. 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. The mean (±S.E) of serum melatonin was determined 63.23±9.51 pg/mL. The concentration of serum melatonin of mares was nearly 2 times than stallions and significantly differed (p = 0.01). Thus serum melatonin related to breed of horses and may affect reproductive activity in different breed and geographical region.

Keywords: HPLC, kermanshah region, kurdish horse, melatonin

INTRODUCTION

Seasonal changes have effects on reproduction in some animals (Clay and Clay, 1992). The pineal gland is able to receive photo information and regulate the secretion of melatonin (Arendt, 2005; Guillaume et al., 2006). Melatonin is a key player in controlling reproduction and circadian rhythm (Baker and Driver, 2007; Gerlach and Aurich, 2000; Keefe and Turek, 1985). 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 (Gerlach and Aurich, 2000; Guerin et al., 1995).

The localization of melatonin receptors in the hypothalamus and pituitary indicates a possible interaction of melatonin in regulation of reproduction in horse. 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 (Nagy et al., 2000).

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 (Guerin et al., 1995). Under natural lighting conditions, serum concentrations of melatonin are higher in anestrus mares during the breeding season (Diekman et al., 2002). 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 gonadotropin hormones and the cessation of ovulatory activity (Fitzgerald and McManus, 2000).

Exogenous melatonin administration is reported to decrease plasma testosterone concentrations in stallions and change the annual reproductive rhythm in pony mares (Peltier et al., 1998). In contrast, artificial photostimulation produced a sharp rise in testosterone levels in stallion (Argo et al., 1991).

After seasonal changes of melatonin in serum of Arabian horses in previously study (Najafzadeh et al., 2011), we decided continue our study on other bred of horses in Iran. Limited information is available about the seasonality of reproductive activity of the Kurdish horse bred in Iran, especially related to melatonin. The aim of the present study was to determine melatonin concentrations in Kurdish horses in Kermanshah region at breeding season.

MATERIALS AND METHODS

Animals: A group of 40 healthy Kurdish mares and stallions, ranged from 10 months to 15 years of age were used. 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) in Kermanshah area of Iran during February to June of 2010. Sera were decanted
immediately into eppendorfs after centrifugation at 3000 rpm for 10 min 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 (Bechgaard et al., 1998; Itoh et al., 1999; Peniston-Bird et al., 1993; Gupta et al., 2006). 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). The mean of serum melatonin was statistically determined using SPSS program and compared by independent t-student test. The minimum level of significance was p<0.05.

**RESULTS**

The mean (±S.E) of serum melatonin of mare was determined 84.84±11.02 pg/mL. This concentration was nearly 2 times than stallions and significantly differed (p = 0.01). Minimum concentration of melatonin of mare was measured at 5.13 pg/mL while maximum concentration was measured at 114 pg/mL. The mean (±S.E) of serum melatonin of stallion was determined 38.91±11.13 pg/mL. Minimum concentration of melatonin of stallion was measured at 1.92 pg/mL while maximum concentration was measured at 100.55 pg/mL. The total mean of melatonin was measured 63.23±9.51 pg/mL (Table 1).

**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 (Diekman et al., 2002). Regulation of reproductive activity via photoperiodic cues mediated by the secretion of melatonin from the pineal gland is reported in ewes (Bittman et al., 1983; Matthews et al., 1993), but the relationship is less clear in mares (Diekman et al., 2002; Kilmer et al., 1982).

Under natural photoperiodic conditions, serum concentrations of melatonin are higher in anestrous mares (Diekman et al., 2002; Sharp et al., 1980). In response to artificial photoperiod, alterations in ovulatory season also exhibit in mares (Freedman et al., 1979; Kooistra and Ginther, 1975). 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 (Murphy et al., 2007). The annual change in photoperiod is considered the primary environmental factor that synchronizes seasonal reproductive activity in mares (Fitzgerald and McManus, 2000). 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 (Fitzgerald et al., 2000); specifically, treatment suppressed the increase in prolactin during spring months.

Information on photoperiod and the physiological reproductive season in Kurdish horse is very limited. Diekman et al. (2002) reported that mean melatonin levels in mares were 16 pg/mL in June and 19.5 pg/mL in December. Guillaume et al. (2006) reported that mean concentration of nocturnal melatonin were 24 pg/mL during autumn in 110 pony mares. Najafzadeh et al. (2011) reported that the season did not significantly affect serum melatonin level in Arabian horses in Ahvaz region. While, Altinsaat et al. (2009) shown the serum concentrations of melatonin were greater in the non-breeding season than breeding season in both mares and stallions. However, Fitzgerald and Schmidt (1995) reported that there is no association between melatonin and reproductive activity in mares during the non-breeding season.

In conclusion, our observations suggested a role for melatonin in the breeding activity in the Kurdish 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 Kurdish horses in Kermanshah area.

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REFERENCES


