

*Full Length Research Paper*

## **Serum testosterone in Arabian stallions during breeding and non-breeding seasons in Iran**

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**The study was conducted to investigate the serum testosterone concentrations of Arabian stallions during breeding and non-breeding seasons under natural photoperiodic condition in west south of Iran (Khuzestan Province; latitude 48:40°N, longitude 31:20°E and altitude 22.5 m). The blood samples (10 ml) were collected from the jugular vein, and it stood at room temperature for 3 h and was centrifuged. The sera were kept at -20°C until assayed by radioimmunoassay. The results show that total serum testosterone levels (ng/ml) in Arabian stallions was higher in May to June (Summer Solstice; 1.08±0.078) than in November to December (Winter Solstice; 0.65±0.11) (p<0.05). We conclude that serum testosterone concentration during the non-breeding season is lower than that of the breeding season. The results confirm a seasonal rhythm in the reproductive cycle of Arabian stallions over the year in this specific region.**

**Key words:** Arabian stallion, season, testosterone, photoperiod.

### **INTRODUCTION**

The horse is a seasonal polyestrous species associated with increase in day light (Guillaume, 1996; Nagy et al., 2000) temperature and availability of food (Nagy et al., 2000). Long day period increases the mean LH and T2 levels in pony stallions and the onset of the ovulatory season in pony mares (Berndtson et al., 1983). The natural breeding season occurs from April to September in the northern hemisphere (Nagy et al., 2000). The seasonal pattern of plasma T2 concentrations in the stallion (Cox et al., 1988) and reduced male reproductive efficiency during the same periods of the year (Boyd et al., 2006; Todini et al., 2007) were confirmed previously. Seasonal changes in day length influence the reproductive activity of many species (Altinsaat, 2009). However, the geographical situations also can affect the reproductive seasonality through changes in photoperiod duration; first, at tropical latitudes below approximately

15°, the annual change in day length is not sufficient to provide a reliable seasonal cue. Second, above 30° latitude, day length provides a strong and highly reliable seasonal cue over evolutionary time. Third, at very high latitudes, animals encounter near-constant light in the summer and near-constant darkness during the winter.

Consequently, animals must have mechanisms to cope with constant day length experienced by migratory animals in the tropics (Bradshaw and Holzapfel, 2007). In Europe, the onset of breeding activity in the male red fox has been related to photoperiod. In high latitudes (>60°N), the maximum fertility is found with increasing day length, whereas at lower latitudes (<50°N decreasing day length is associated with fertility. Reproductive activity of the male red fox increased during long days in high latitudes (> 60°N) and decreased in short day period of year in lower latitudes (< 50°N) (Boyd et al., 2006), while the ewes were seasonally polyestrous at higher latitudes (> 35°) (Rosa and Bryant, 2003). According to Guillaume (1996), fertility in stallions may be maintained at a reduced level outside the breeding season. On the other hand, latitudes between 30 and 40°N do not exhibit

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the same dramatic seasonal changes in reproductive activity as animals from higher latitudes.

Nevertheless, no data exist on seasonal variation in semen quality in horses from those latitudes (Gamboa et al., 2010). Limited information is available about the seasonal reproductive activity of the Arabian horse in Khuzestan. The aim of the present study was to determine the serum concentrations of T2 in Arabian stallions, during breeding and non-breeding seasons under natural photoperiodic condition.

## MATERIALS AND METHODS

### Animals and location

The experiment was carried out in Khuzestan Province of Iran in Ahvaz (latitude 48:40°N, longitude 31:20°E and altitude 22.5 m) with an average annual rainfall of 15.48 mm (range: 0 to 44.8 mm) and temperature range: 1 to 50°C. Healthy Arabian stallions (n=23) with age range of 3 to 15 years and weight range of 450 to 600 kg were arranged in a changeover design experiment and examined twice in May to June [June 21(Summer Solstice): 14 h 11 min of light (L): 9 h 49 min of darkness (D)] and November to December [December 21(Winter Solstice): (10 h 26 min L: 13 h 24 m D)] 2008 to 2009. The animals were fed with the standard adult horse diet and housed in the stable (40 and 8°C in Summer and Winter, respectively). The experiment was conducted under natural photoperiod.

### Blood collection

Blood samples (10 ml) from all stallions were collected via the jugular vein during summer solstice (May to June) and Winter Solstice (November to December). All samples were transported to the laboratory on ice. The blood serum was separated by centrifugation at 3000 rpm for 10 min and stored at -20°C until assayed.

### Hormone analysis

The serum T2 concentration was measured using commercial Radioimmunoassay (RIA) kits (TESTO-RIA-CT, BioSource Europe SA, 8, Rue de l'Industrie\_1400-Nivelles\_Belgium). The minimum detectable T2 concentrations were 0.05 ng/ml. The standard range was 0.1 to 17 ng/ml for testosterone assays and the intra-assay coefficient of variation (CV) was 4.1%. All samples were run in duplicate and within one assay.

### Statistical analyses

Mean serum concentration of T2 was compared with paired t-test using SAS. Mean environmental temperature, relative humidity and total solar lighting were compared between two seasons using t-test. Data was expressed as LSmean±SEM.

## RESULTS

The meteorological data (Figure 1) indicated a significant difference between Summer and Winter in total lightness

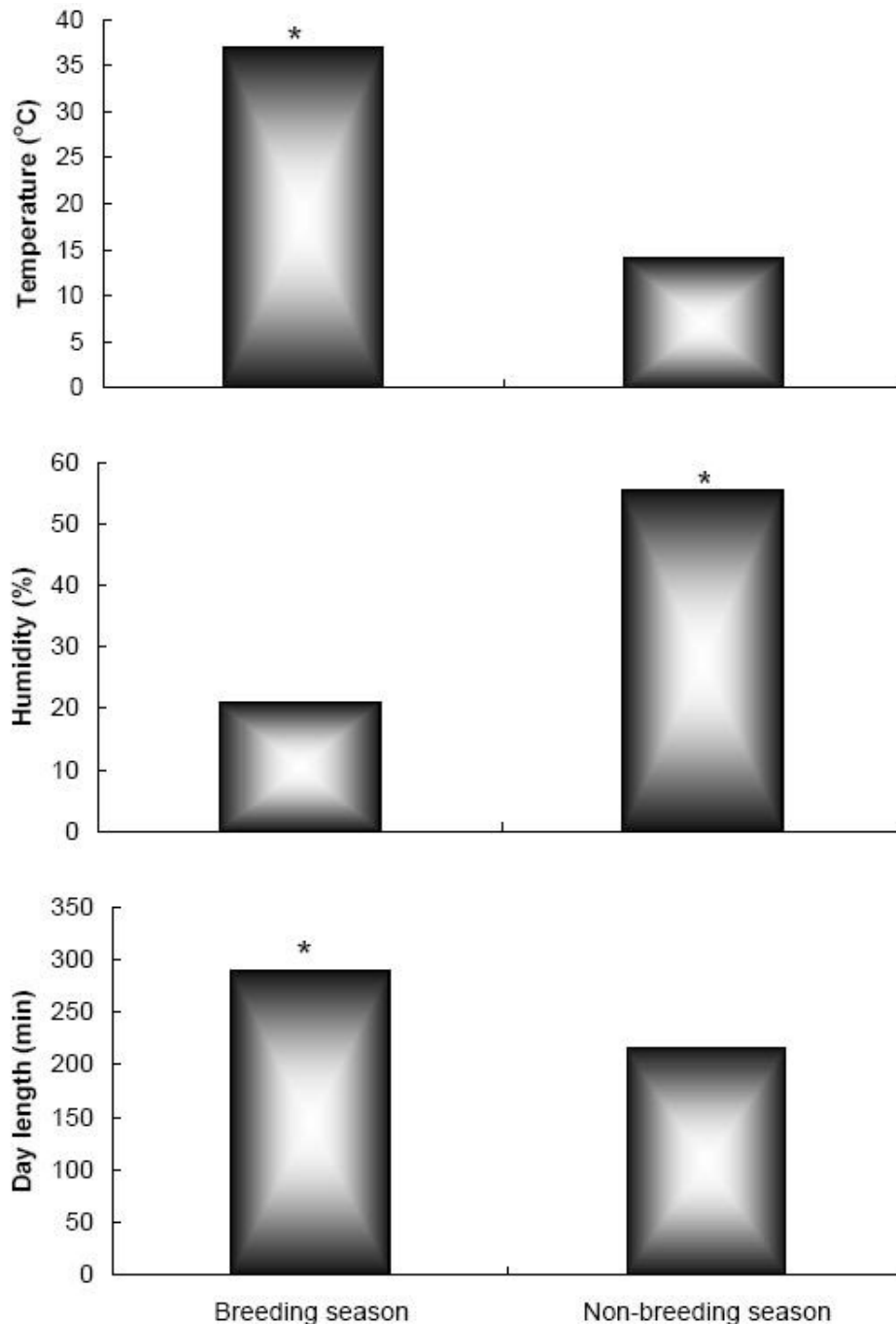
times (836.7±1.6 vs. 620.7±1.7 min, respectively) and ambient temperature (38.4±0.33 vs. 9.7±0.8°C). Total serum T2 levels (ng/ml) in Arabian stallions were 1.08±0.078 and 0.65±0.11 ng/ml in May to June (summer solstice) and November to December (Winter Solstice), respectively (p<0.05).

## DISCUSSION

The results of the present study clearly showed a seasonal rhythm in reproductive cycle of Arabian stallions over the year in this specific region. These results may confirm no impact of the stressful environmental conditions on the reproductive function of Arabian stallions. Photoperiod controls the reproductive cycle of some species including horse, goat, sheep and hamster (Altinsaat et al., 2009; Noakes et al., 2001; Cunningham and Bradley, 2007). In the northern hemisphere, mares enter anestrus and stallions semen quality sharply declines during late fall and early Winter (Altinsaat et al., 2009). Light signals are received by the retina and transmitted to the pineal gland which produces melatonin in response to darkness. Melatonin plays a key role in regulation of seasonal reproductive activity (Fitzgerald et al., 2000; Rosa and Bryant, 2003; Cunningham and Bradley, 2007; Nagy et al., 2000; Gerlach and Aurich, 2000). Melatonin regulates GnRH in a negative fashion (Roster, 2008; Nagy et al., 2000; Gerlach and Aurich, 2000). Normally, GnRH regulates the release of LH and FSH from the anterior pituitary. LH in turn regulates the steroidogenic pathway in testes and stimulates Leydig cells to produce testosterone in the adult males (Roser, 2001, 2008).

In high latitude (> 30°), day length has impact on the reproductive activity of seasonal breeders (Bradshaw and Holzapfel, 2007). The results of the present study confirmed the previous studies on Arabian stallions (Altinsaat et al., 2009) and Welsh mountain pony stallions (Cox and Skidmore, 1991) in which higher plasma T2 concentration during breeding season was recorded than in the non-breeding season in the northern hemisphere.

Altinsaat et al. (2009) reported that at latitude 39:46°N and longitude 30:32°E in the region, the mean concentration of T2 of Arabian stallions were 6.58±0.50 and 3.64±0.48 ng/ml under natural photoperiodic conditions during breeding and non-breeding seasons, respectively. The highest plasma T2 concentrations of Welsh mountain pony stallions in Britain was reported in the Spring (April and May) and the lowest concentrations was reported in December to February (Cox and Skidmore, 1991). They reported a steady state increase of plasma LH and T2 concentrations from January (8L:16D) to July (16L:8D) under natural daylight; although, artificial photo-stimulation can make a sharp rise in plasma T2 levels in stallion (Altinsaat et al., 2009). Cox et al. (1988) reported that illumination of stallions



**Figure 1.** Three different environmental parameters (temperature, relative humidity and day length) at two time point of Winter and Summer seasons (Lsmeans±SEM). \* Indicates statistical significance between values with  $p \leq 0.05$ .

with 16.5 h of light/day from 21 June to mid December which resulted to maximum T2 concentrations in October. The lighted stallions (16 h light/day, December 15 to April 1) appeared to become refractory to the lighting program, since both testicular size and plasma T2 concentrations were significantly reduced by June (Nagy et al., 2000).

Plasma concentrations of LH and testosterone increased under an artificial photo-stimulation during the Winter in stallions (Berndtson et al., 1983). The higher levels of plasma T2 concentrations during the breeding season compared to the non-breeding season, may relate to increased testicular perfusion (Boyd et al., 2006) or

decreased inhibitory effect of melatonin on GnRH pulses (Altinsaat et al., 2009).

The effects of photoperiod on reproduction can be modified to a certain extent by temperature, nutrition (Langford et al., 1999; Gerlach and Aurich, 2000), body condition or age (Gerlach and Aurich, 2000). The meteorological data in the present study showed a significant increase in mean environmental temperature in June than in January. The previous report showed an impact of heat stress on testicular structures specially germ line cells with no adverse effect on T2 profile of ram lambs (Rasooli et al., 2010). Higher levels of T2 in the present study in June, in spite of heat stress, may confirm that testicular endocrine function in Arabian stallions is not affected by heat stress. However, scrotal heat insulation decreased sperm quality in stallions (Love and Kenney, 1999). Evaluation of the semen parameters was not in the scope of the present study.

## Conclusion

The present data extend this conclusion by showing that photoperiod is a major environmental factor of regulation of T2 secretion in Arabian stallion.

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