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Alterations of circulating cortisol, IGF-1, and thyroid hormones in Darehshori horses during a year

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Abstract: Season may be considered as a factor that affects the equine endocrine profile. Hence, from January to November 2015, 27 clinically healthy adult Darehshori racehorses were selected. Thirteen of them were stallions and the others were mares. Ten of them were more than 10 years old and 17 horses were under the age of 10 years. None of them were pregnant or lactating during the study. Body condition scores of 12 horses were below and the others were above 5. Blood samplings and body condition score evaluations were performed every 45 days during a year. Serum levels of cortisol, IGF-1, T3, T4, and TSH were assayed in all specimens. T3 and TSH were significantly decreased following the warming up of the climate in different ages, sexes, and body condition scores, but there were no significant changing patterns in T4. There were various trend changes of both cortisol and IGF-1 in different ages, sexes, and body condition scores during the seasons of the study period. Based on the results, circulating levels of cortisol, IGF-1, and thyroid hormones of Darehshori horses are dependent on many factors such as breed, sex, age, body condition, season, geographical location, and individual characteristics.

Key words: Season, age, sex, body condition score, endocrine profile, Darehshori horses

1. Introduction

There are several concepts about physiological alterations in the body in various seasons and several theories have explained these mechanisms, but the mechanisms are not clear in various seasons. Furthermore, several surveys have demonstrated that hormones may play an important role in these seasonal alterations, or vice versa, based on changes in temperature and light. According to available evidence, there is no information on changes in cortisol, insulin-like growth factor-1 (IGF-1), and thyroid hormones in Darehshori horses in different seasons. Therefore, the present study investigated the seasonal changes in the mentioned hormones in both sexes of Darehshori horses (1,2).

Cortisol is a hormone synthesized by the adrenocorticotropic hormone (ACTH) produced in the pituitary gland in response to stress, which also increases blood glucose levels. Cortisol secretion occurs in the body's reaction to stress, and because it is responsible for several stress-related changes in the body, it is also called stress hormone. This hormone has several effects on inflammatory reactions, blood glucose metabolism, release of insulin hormone to maintain the proper level of blood glucose, body defenses, and regulation of blood pressure. It is also increased during stress in a horse, which counteracts glucose metabolism by increasing stress; however, chronic stress and high cortisol levels for prolonged periods can lead to problems such as aggressive behaviors, reduced fertility, possibility of immune suppression, and increased incidence of gastric ulcers, colitis, and diarrhea (3).

IGF-1 is a protein with a similar insulin structure that is effective in the growth and metabolism of many cells. IGF-1 is made by the liver by stimulating growth hormone and it is effective in cell metabolism and cell growth. IGF-1 is an anabolic hormone that increases muscle volume and decreases body fat, and it facilitates the transfer of amino acids and glucose, supports the positive balance of nitrogen and glycogen synthesis, and has anabolic effects in bone and cartilage.

Thyroid hormones are derived from tyrosine amino acids. Approximately 95% of the hormones secreted from the thyroid gland is thyroxine (T4). Although the level of triiodothyronine (T3) released from the thyroid gland is negligible, this hormone plays a major role in the effects of thyroid hormones on the body. The main part of blood T3 is from the conversion of T4 to T3 in peripheral tissues

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such as the liver, kidneys, and placenta. Several functions have been described for thyroid hormones in the body, such as increasing the rate of metabolism and glucose uptake from the digestive system, stimulating the process of glucose recovery, enhancing the effect of catecholamines and insulin, increasing the cardiac output, and sometimes increasing cardiac muscle contraction (3).

Thyroid hormones, in addition to regulating temperature and energy and protein metabolism, play a role in regulating some ovarian activities. On the other hand, their serum concentrations are significantly affected by age, sex, race, and ambient temperature. Research has been done on the effects of various physiological conditions such as pregnancy and lactation in cattle and sheep on changes in thyroid hormones. It has been shown that the levels of these hormones in each physiologic period are different from other courses (4,5).

Considering the importance of cortisol, IGF-1, and thyroid hormones in the growth and immune system of the horse's body, as well as many other functions that are proposed for these hormones, the aim of this study was investigation of the serum levels of these hormones in different sexes, ages, and body conditions among Darehshori horses in various seasons during a year. The results of this study can explain the possible effects of the above-mentioned variables on changes in these hormones in Darehshori horses. The results of the present research may be used as an indicator for determining the natural and abnormal levels of these hormones in Darehshori horses in each sex, season, and body condition to assess metabolic and physiological disturbances.

2. Materials and methods

2.1. Study population

From January to November 2015, 27 adult Darehshori racehorses were selected from two horse rearing centers in Shiraz, a city in southwestern Iran. Thirteen of them were stallions and the others were mares. Ten of them were more than 10 years old and 17 horses were under the age of 10 years. Before initiation of the study, the clinical status of the horses was examined and their health was clinically confirmed. Common antiparasitic prevention was also administered to all horses. Animals were kept in solitary places and had easy access to water and food, and their diet was based on the usual planning in each rearing center, which was similar in both. They were fed with balanced rations including alfalfa hay, corn silage, and barley grain. None of them were pregnant or lactating during the study.

2.2. Blood sampling and endocrinological studies

Blood samples were collected via jugular venipuncture from all animals every 45 days in plain tubes. Air temperature (°C) and day/night length (h) of Shiraz (29.5918°N, 52.5837°E) at each sampling point during the study are presented in the Table. The first sampling was carried out on 1 January 2015 and samplings continued to 15 November 2015. Immediately after blood collections, sera were separated by centrifugation for 10 min at 3000 × *g* and stored at –22 °C until assayed.

Circulating cortisol levels were determined by RIA kit (Institute of Isotopes Co., Ltd., Hungary) and concentrations of IGF-1 were evaluated by equine-specific ELISA kits (Eastbiopharm, China). Thyroid-stimulating hormone (TSH) was measured by equine ELISA kits (Eastbiopharm). Serum T3 concentrations were determined using a competitive enzyme immunoassay kit (Padtan Elm Co., Iran). Serum T4 concentrations were measured using a competitive enzyme immunoassay kit (Monobind Inc., USA).

2.3. Evaluation of body condition scores

Veterinarians use a series of criteria to evaluate the rate of obesity and slimming in horses, and according to these

Table. Air temperature (°C) and day/night length (h) of Shiraz (29.5918°N, 52.5837°E) on each sampling day during the study.

Days	Temperature (°C)		Day/night length (h:min)	
	Minimum	Maximum	Day	Night
1 January 2015	5	19	12:01	11:59
15 February 2015	8	14	12:45	11:15
30 March 2015	7	24	14:00	10:00
15 May 2015	12	32	15:25	08:45
30 June 2015	19	39	16:04	07:56
15 August 2015	20	38	14:56	09:04
30 September 2015	14	32	13:30	10:30
15 November 2015	1	22	12:20	11:40

criteria, they give the body condition of the horses. Body condition scores of the animals were estimated based on Henneke et al. (6) with a 0 to 9 scoring system. In this system, six parts of the horse's body are first observed and then touched and given a physical score according to the physical condition of the horse. For these six points, the probability of accumulation of adipose tissue is higher than in other parts of the horse's body, and they include the neck blade, the back of the shoulder joint, the ribs, the tail, and the back of the waist. Body condition scores of 12 horses were under and the others were above 5. The horses were evaluated every 45 days during a year and body condition scoring was performed each time.

2.4. Statistical analysis

Data are presented as mean ± standard deviation. Differences between the average concentrations of each hormone between groups (age, sex, and body condition score) on similar days were analyzed by two independent samples t-test. Repeated measures ANOVA was used to evaluate the seasonal alterations of each hormone during the year. Statistical analyses were performed using SPSS 11.5 for Windows (SPSS Inc., USA). The level of significance was set at $P < 0.05$.

3. Results

Changes in cortisol, IGF-1, and thyroid hormones in different sexes, ages, and body conditions during a year in Darehshori horses are presented in Figures 1–5.

3.1. Thyroid hormone alterations

The lowest T3 in the young horses was detected in June and its lowest levels in aged horses were seen in the August. The highest levels of this hormone were observed in both groups in February. In both age groups, T3 decreased following the warming up of the climate, and this decrease was more pronounced in the aged horses (Figure 1).

There were no significantly changed patterns of T4 in different ages, sexes, and body condition scores during the study period. The highest T4 in young and old horses was observed in June and February, respectively. In both age groups, the lowest T4 levels were detected in February. The results of repeated measures ANOVA showed that the concentration of T3 in both old and young horses decreased significantly with the warming of the climate and in general, while T4 changes in both age groups during the study were not statistically significant ($P > 0.05$).

In both lean and obese horses, the lowest levels of T3 were seen in August. Following the warming of the climate, the concentration of T3 declined in all studied horses. In lean and obese horses, the highest amounts of T4 were seen in January and September, respectively. In addition, the lowest amount of this hormone in lean horses was found in both February and September, and in obese horses it was detected in February. The results of repeated measures ANOVA demonstrated that T3 concentration in both lean and obese groups decreased significantly with the warming of the climate ($P < 0.05$; Figure 1).

T3 concentrations in mares were significantly higher than in stallions in all sampling stages during the year (Figure 1). The highest levels of this hormone in mares and stallions were seen in February and January, respectively. The lowest amount of T3 was observed in stallions in August and in mares in June. The levels of this hormone in both sexes in warmer seasons were significantly ($P < 0.05$) lower than in other seasons, and these changes were more pronounced in stallions then mares (Figure 1). There were no significant differences between T4 levels in both sexes during the study period ($P < 0.05$; Figure 2). The highest values of T4 were observed in May in both sexes. Moreover, the lowest serum levels of this hormone were detected in June in both mares and stallions. The results of repeated measures ANOVA showed that the T3 concentrations in both sexes decreased significantly with the warming of the climate ($P < 0.05$; Figure 1).

Changing patterns of TSH for all studied parameters (age, sex, and body condition score) were detected significantly and this factor was decreased significantly with the warming of the climate ($P < 0.05$; Figure 3). Serum TSH levels in stallions, aged horses, and obese horses were significantly lower than in mares, young ones, and lean ones during the study period (Figure 3). The lowest levels of TSH in stallions and mares were seen in June and both August and September, respectively. The highest values of this parameter in stallions were in January and in mares were in both January and February. The lowest levels of TSH in aged, lean, and obese horses were observed in September. The highest serum concentrations of this factor in young and obese horses were detected in March.

3.2. Cortisol alterations

In all stages of sampling, serum cortisol concentrations in mares were significantly higher than those of stallions ($P < 0.05$; Figure 4). The highest and lowest serum levels of this hormone in mares were seen in January and November, respectively, while in the stallions the highest and lowest levels of the hormone were observed in February and September, respectively. In all samples, serum levels of cortisol in obese horses were significantly higher than others ($P < 0.05$; Figure 4). In both obese and lean groups, the highest and lowest levels of this hormone were seen in January and November, respectively. Generally, serum cortisol concentrations in older horses (more than 10 years old) were significantly higher than in younger ones (younger than 10 years) ($P < 0.05$; Figure 4). In elderly horses, the highest and lowest serum cortisol levels were seen in February and May, respectively. Also in young horses, the highest and lowest concentrations of this hormone were seen in May and February, respectively.

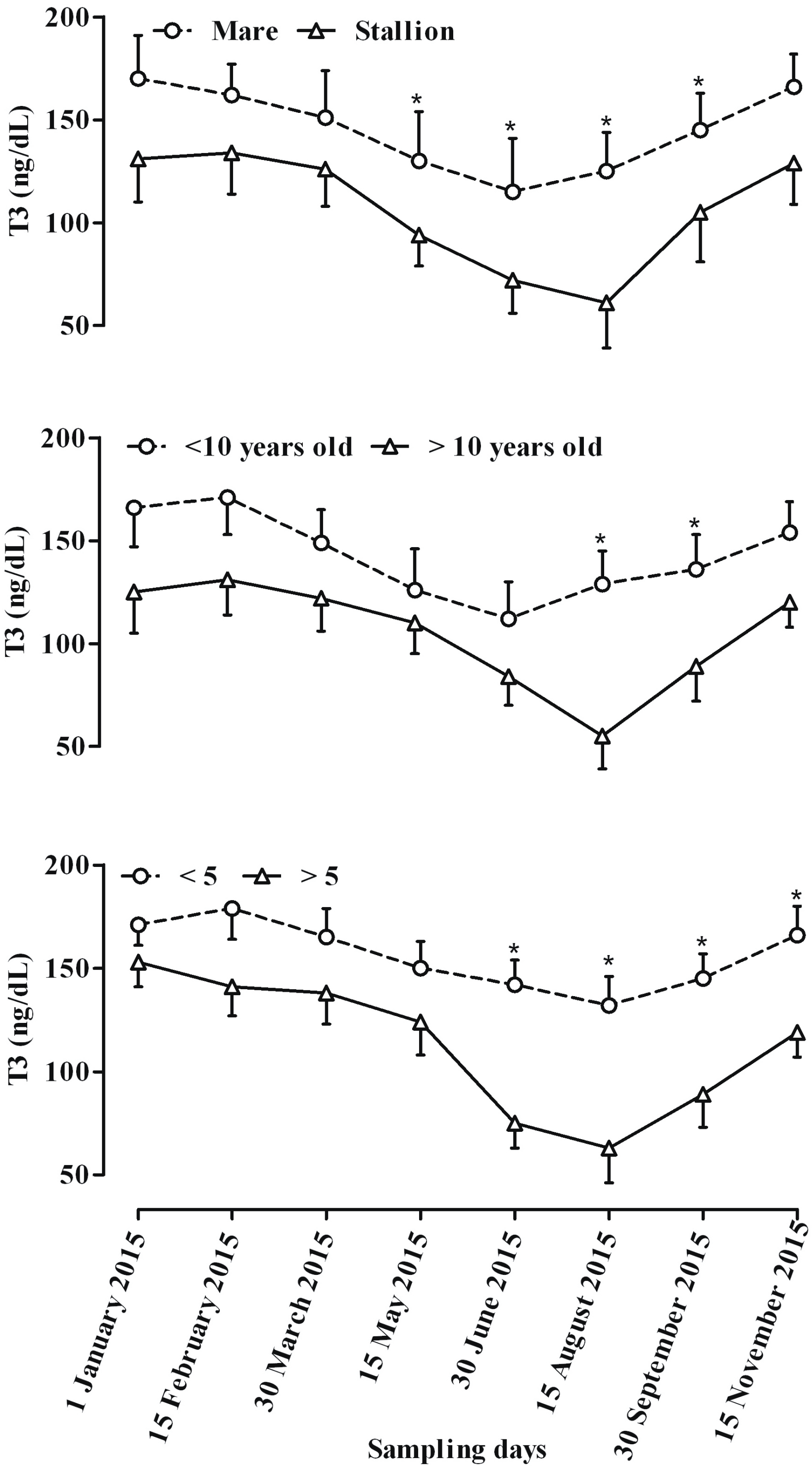

Figure 1. Seasonal alterations of circulating T3 (mean ± SD; ng/dL) in different sexes (stallion/mare), ages (older/younger than 10 years old), and body condition scores (above/below 5) of Darehshori horses. Asterisks indicate significant differences between two groups on the same sampling day (P < 0.05).

3.3. IGF-1 alterations

In all stages of sampling, serum concentrations of IGF-1 in mares were significantly lower than in stallions (P < 0.05; Figure 5). In both sexes, the highest and lowest serum levels of this hormone were observed in February and September, respectively. In all samples, serum levels of

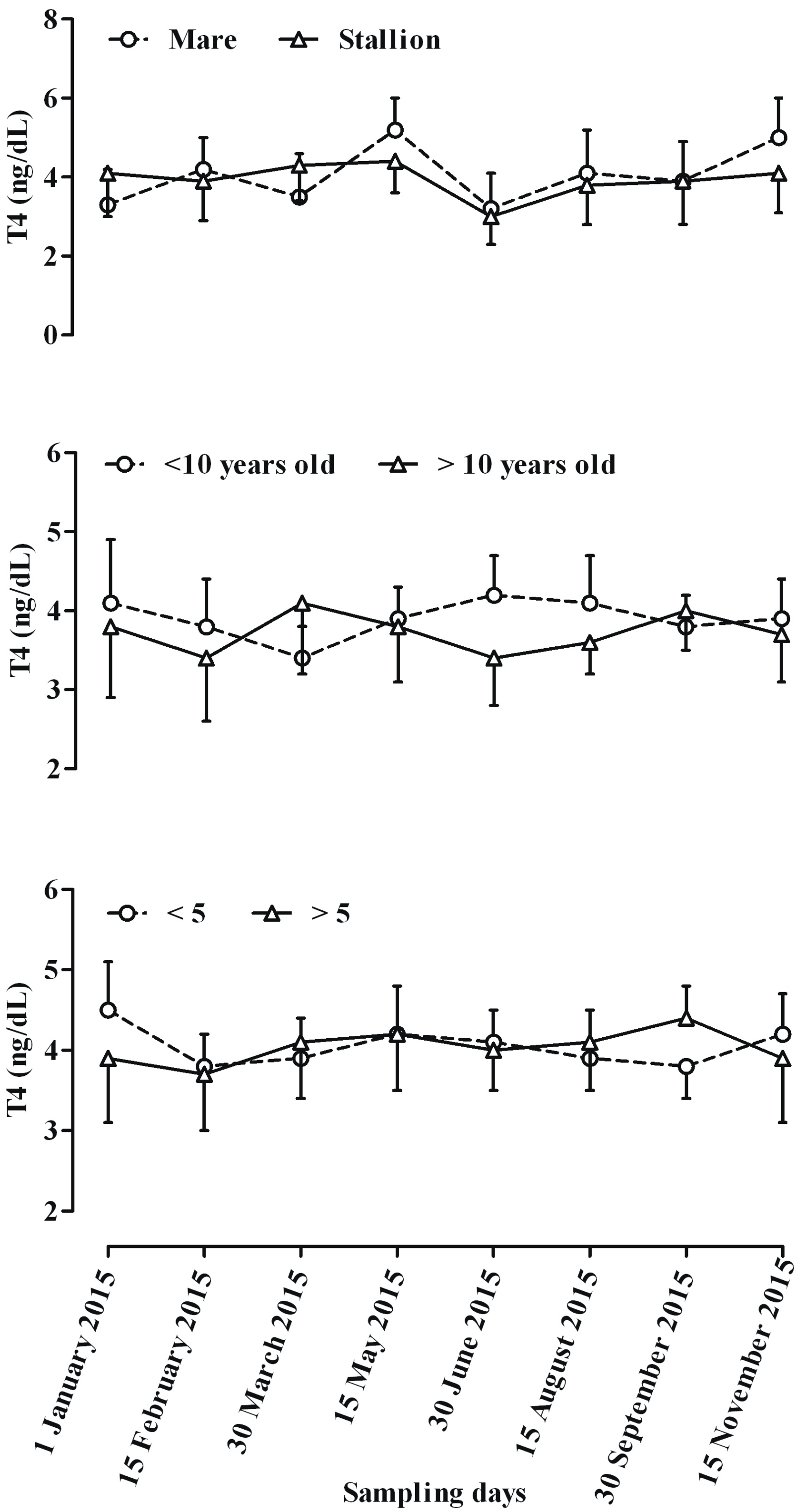

Figure 2. Seasonal alterations of circulating T4 (mean ± SD; ng/dL) in different sexes (stallion/mare), ages ((older/younger than 10 years old), and body condition scores (above/below 5) of Darehshori horses. Asterisks indicate significant differences between two groups on the same sampling day ($P < 0.05$).

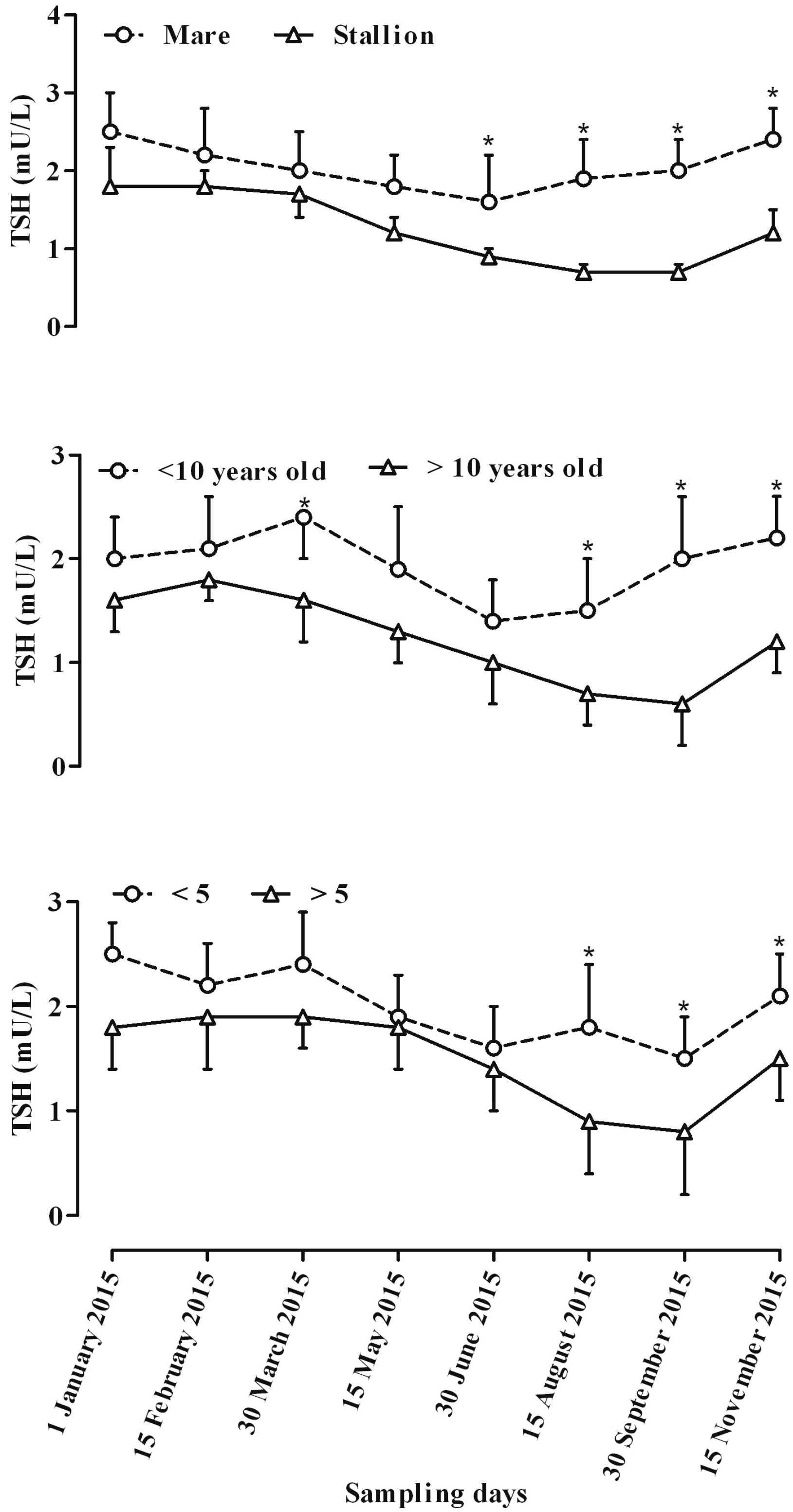

Figure 3. Seasonal alterations of circulating TSH (mean ± SD; mU/L) in different sexes (stallion/mare), ages ((older/younger than 10 years old), and body condition scores (above/below 5) of Darehshori horses. Asterisks indicate significant differences between two groups on the same sampling day (P < 0.05).

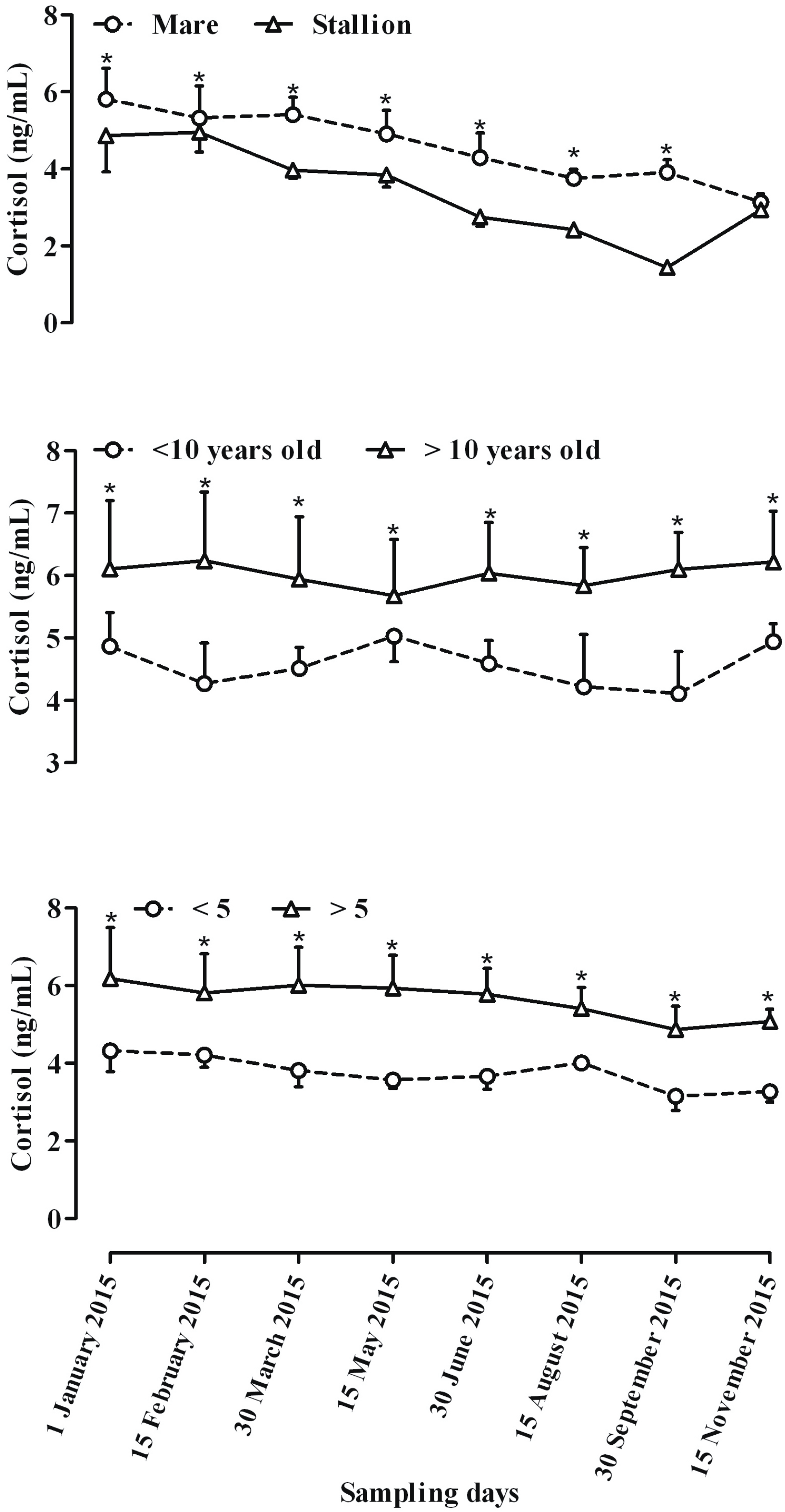

Figure 4. Seasonal alterations of circulating cortisol (mean ± SD; ng/mL) in different sexes (stallion/mare), ages ((older/younger than 10 years old), and body condition scores (above/below 5) of Darehshori horses. Asterisks indicate significant differences between two groups on the same sampling day (P < 0.05).

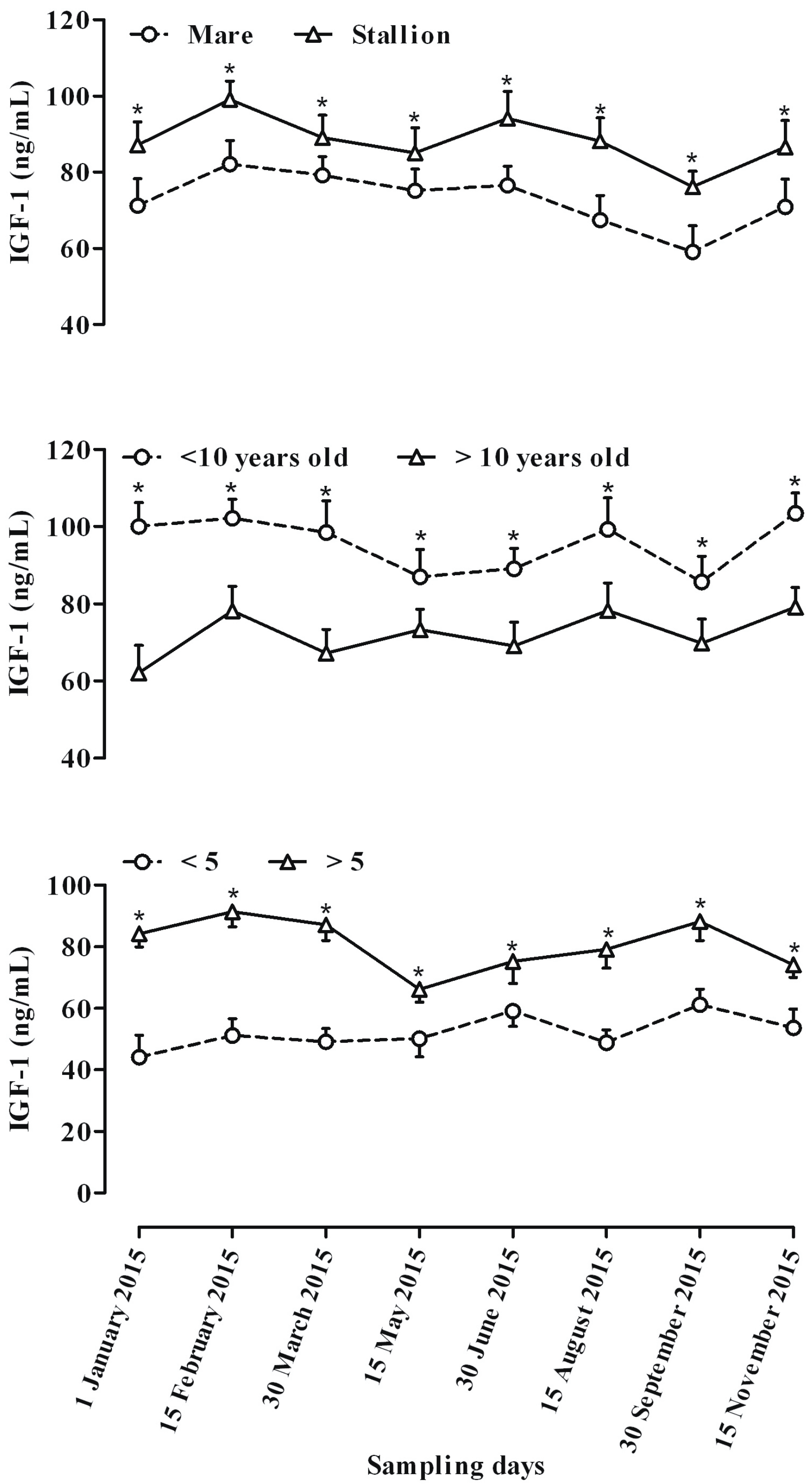

Figure 5. Seasonal alterations of circulating IGF-1 (mean ± SD; ng/mL) in different sexes (stallion/mare), ages ((older/younger than 10 years old), and body condition scores (above/below 5) of Darehshori horses. Asterisks indicate significant differences between two groups on the same sampling day ($P < 0.05$).

IGF-1 in obese horses were significantly higher than others ($P < 0.05$; Figure 5). In horses with better body condition scores, the highest and lowest levels of this hormone were seen in February and May, respectively. Moreover, in the lean group of horses, the highest and lowest serum levels of IGF-1 were observed in September and January, respectively. Generally, serum IGF-1 concentrations in young horses (less than 10 years old) were significantly higher than in older ones (more than 10 years old) ($P < 0.05$; Figure 5). In young horses, the highest and lowest serum levels of IGF-1 were seen in November and May, respectively. In addition, in the older horses, the highest and lowest concentrations of this hormone were seen in November and January, respectively.

4. Discussion

4.1. Thyroid hormone alterations

There are several concepts about the whole body's physiological alterations in different seasons, and various theories are proposed to explain these mechanisms. In the literature it has been mentioned that thyroid hormones may play an important role in the whole body's physiological changes during a year, or vice versa, based on changes in temperature and light (7). According to available evidence, there is no information on thyroid hormone alterations in Darehshori horses in different seasons. Therefore, one of the aims of the current research was investigation of the seasonal effects on thyroid hormones in different sexes, ages, and body condition scores of Darehshori horses. The results of this study can be used as an indicator for determining natural and abnormal levels of thyroid hormones in this breed, which can be used to evaluate metabolic and physiological disorders.

Small amounts of circulating T3 and T4 cause low metabolic rates in horses, and these concentrations may be due to acquired problems or secondary hypothyroidism due to reduced TSH secretion from the pituitary gland. However, some evidence suggests that many of the clinical symptoms associated with hypothyroidism in horses are part of the symptoms of metabolic syndrome, and researchers have found that thyroid hormone supplementation is effective in treating this syndrome (8). The results of the present study showed that concentrations of T3 were related to levels of TSH (Figures 1 and 3), such that decreased TSH levels were aligned with decreased T3 concentrations.

In relation to the effects of the season, several environmental factors such as temperature, daytime, heat intensity, wind speed, radiation, and humidity affect changes in thyroid hormones that may affect thyroid functions (9). In experiments conducted by Ingraham et al. (10) in cows, the level of thyroid hormones in the blood had a negative correlation with the environmental temperature and humidity (10). Some researchers reported high thyroid activity in cows in cold weather (11). According to a study by Mixner et al. (12), the months and seasons have a greater effect on thyroid hormone secretion than pregnancy stage. The level of T4 secretion in cows was highest and lowest in the spring and autumn, respectively (13).

In the study of Gooraninejad et al. (14) on Arab horses in Khuzestan, Iran, it was concluded that serum T3 and T4 concentrations were significantly affected by the season; the highest concentration of both hormones was seen in winter and the lowest in summer. Maddahi et al. (15) studied Arab stallions and reported that the function of their thyroid glands is partly dependent on seasonal variations and age. In a study of Arab horses by Ghadrdan Mashhadi et al. (16), they concluded that serum concentrations of T3 and T4 were higher in the cold season.

Exposure to cold weather activates the pituitary–thyroid system by several mechanisms and stimulates TSH in 10–30 min. The activation of TSH secretion is associated with increased thyroid activity. The first trigger to activate this system is an increase in the release of thyrotropin-releasing factor (TRF) from the hypothalamus. If the environmental temperature decreases, TSH gradually increases 90 min after the onset of cooling (17). Increased TSH secretion in animals exposed to cold is due to the increased release of TRF from the hypothalamus. However, TRF is not only distributed in the hypothalamus and it has been found in several tissues at high concentrations, but nothing is known about the potential role of TRF in these tissues in regulating TSH secretion (17). In the present study, the T3 concentration decreased due to the warming of the climate. As mentioned earlier, air cooling is an important factor to stimulate the thyroid gland in secretion of thyroid hormones, and the results of this study confirm this phenomenon.

A study by Eswari et al. (18) on sheep suggested that thyroid hormones decreased during aging. Conversion of T4 to T3 also decreases in aged animals, resulting in a significant decrease in T3 of the plasma. Therefore, it seems that thyroid gland activity decreases with age. Another study on sheep by Maharajan et al. (19) stated that the metabolic rate in young animals is higher than in adults, and the role of thyroid hormones alongside growth hormone is also evident in the growth process. In the present study, the concentration of T3 hormone in young horses, in all seasons, was higher than in older ones.

Studies by Eswari et al. (18), Emery et al. (20), and Prasad (21) suggested that thyroid hormone levels in females were higher than in males, indicating that thyroid activity is higher in female animals. In our study, although the concentration of T3 hormone in the mares in all seasons was numerically higher than in stallions, this was statistically significant only in May, June, August, and September ($P < 0.05$; Figure 1).

Ghorbani et al. (22) investigated the serum concentration of various mineral elements and thyroid hormones in Caspian horses of different nutritional conditions, ages, and sexes. They concluded that serum concentrations of TSH and T3, fT3, T4, and T3/T4 ratio in mares were significantly higher than in stallions. In their study, it was concluded that the effects of age and diet on thyroid hormones were not significant (22). In a study by Nazifi et al. (23), T3 and T4 concentrations in Turkmen stallions were significantly lower than in mares. In addition, the concentration of T3 in obese horses, especially in warm seasons, was lower than in lean horses. The possible causes of this finding are the lower metabolic rate of obese horses compared to lean ones. Furthermore, obese horses are susceptible to equine metabolic syndrome (24), and in such horses, the concentration of thyroid hormones is lower than in other horses; hence, thyroid hormone supplementations are recommended to treat equine metabolic syndrome (24).

According to the results of this study, there were no significant differences in the concentrations of T4 in any of the seasons, ages, sexes, or body conditions. One of the probable reasons for this finding is that the T3 is the main hormone of the thyroid gland's activities. Furthermore, T4 in many cases is also converted into T3. However, specific characteristics and physiologic situations of the Darehshori breed may also be attributed to this finding.

4.2. Cortisol alterations

Cortisol is the main hormone associated with stress, which is produced by the adrenal gland. This hormone is the main glucocorticoid secreted by the adrenal gland in stressful conditions and is also involved in regulating blood glucose (25). During the life of animals, there are several stresses that affect growth and reproduction, and the chemical mediator of these stresses is cortisol (26). Aurich et al. (27) reported that control of stressors decreases the cortisol concentrations.

In the present study, serum cortisol concentrations were lower in some months, and its levels in September were commonly lower than in other months (Figure 4). In this study, higher serum cortisol concentrations were detected in winter. One of the reasons for this increase is the stressful conditions after the dropping of the temperature. Moreover, desirable weather conditions, not being in the reproductive season, estrous behaviors, mating, parturition, and lactation, which may be considered as nonstressful conditions, may decrease the circulating cortisol levels in the autumn. Hart et al. (28) studied the effect of age, season, body condition, and endocrine status on free cortisol and insulin concentrations in horses and reported that cortisol levels were higher in older and obese horses. They also observed an increase in cortisol concentrations in the winter compared with autumn. The effect of the season on the hypothalamus–pituitary–adrenal axis is explained in horses. Hart et al. (28) found that ACTH and α-melanocyte-stimulating hormone could increase cortisol concentrations in autumn.

Aurich et al. (27) reported that cortisol levels follow a circadian rhythm and mentioned that their levels in the mornings were higher than at night. Salivary cortisol in the late pregnancy of mares was markedly lower than in parturition. Increasing cortisol levels in stallions during the reproductive season in April and May show a chronic and moderate stressful condition in these animals. In general, that study found that cortisol variations were seasonal. Greenspan et al. (29) stated that serum cortisol concentrations in older horses were significantly higher than in younger ones.

In this study, serum cortisol concentration in obese horses was significantly higher than in lean horses during the study period (Figure 4). Hart et al. (28) argued that obesity can act as an initial stimulus for the hypothalamus–pituitary–adrenal axis and is effective in increasing cortisol levels. Fatty tissues can convert the inactive cortisol (cortisone) to its active metabolite via the beta-11 hydroxy steroid dehydrogenase enzyme, which results in an increase in intratissue and intracellular cortisol. Obese horses mostly suffer from metabolic syndrome, type 2 diabetes, and several endocrinopathies, which may be a reason for higher serum cortisol concentrations in obese horses (24). In aged horses, the higher level of serum cortisol is due to the increased activity of the hypothalamus–pituitary–adrenal axis (30), and the present study showed that the serum cortisol levels in elderly horses were significantly higher than in younger ones during the study period (Figure 4).

4.3. IGF-1 alterations

IGF-1 is an underlying factor that stimulates the growth, differentiation, and function of many cells (31). In the present study, serum concentrations of IGF-1 in younger horses were significantly higher than in elderly ones ($P < 0.05$; Figure 5). The liver synthesis of this hormone was stimulated with androgens and estrogens; thus, IGF-1 can increase during puberty in stallions between 2 and 4 years old (31). Malinowski et al. (1) evaluated the serum levels of T3, T4, IGF-1, and IGF-binding protein in different ages of horse. They observed that IGF-1 and IGF-binding protein affected horse growth. The effects of exercise, age, and sex on IGF-1 were evaluated in horse and results revealed that IGF-1 concentration was more stable in horse compared to growth hormone concentrations. Noble et al. (32) mentioned that the concentration of IGF-1 gradually decreased along with aging and its level in mares was lower than in stallions. Investigating the concentration of IGF-1 on the structure of articular cartilage of Thoroughbred horses at different ages showed that high levels of IGF-1 were detected in 225 days and it was decreased 450 days after puberty. IGF-1 reached a constant level along with the formation of osteochondral tissue (33). Corpas et al. (34) showed that reduced secretion of growth hormone is one of the strongest reasons for decreasing plasma IGF-1 during aging. Carlson et al. (35) found that plasma IGF-1 decreases with age due to a reduction in the growth hormone pulse.

In the present study, serum levels of IGF-1 in stallions were significantly higher than in mares ($P < 0.05$; Figure 5). Clapper et al. (36) measured the serum concentrations of IGF 1, 17-beta estradiol, IGF-binding protein, and testosterone in pigs and observed that the growth hormone receptors in males were higher than in females (36). Previous studies have shown that IGF-1 concentrations are higher in males (2,32).

Champion et al. (2) examined the plasma concentration of IGF-1 in Standardbred horses at rest period. They sampled horses 1–8 years old from five geographic regions of New Zealand. They found that serum levels of IGF-1 in various geographical regions were significantly different, and the serum levels of this hormone in stallions were higher than in mares.

The results of the current study showed that the circulating IGF-1 in obese horses was higher than in lean ones, which is consistent with earlier findings (37). Sticker et al. (37) demonstrated that nutrition (especially the amounts of protein and energy) and body condition score have a great influence on IGF-1 concentration. They investigated the effect of food protein and energy limitations on IGF-1, prolactin, cortisol, and thyroid hormones in mares and found that IGF-1 concentrations in obese females were higher than in lean ones.

4.4. Conclusions

Based on the results of the current research and other previous studies, circulating levels of cortisol, IGF-1, and thyroid hormones are dependent on many factors. It may be stated that factors such as breed, sex, age, body condition, season, geographical location, and individual characteristics of horses may affect the levels of these hormones. Furthermore, the results of this study may be used as a guideline to better understand the endocrinological concepts of Darehshori horses in relation to season, age, sex, and body condition score.

References

1. Malinowski K, Christensen RA, Hafs HD, Scanes CG. Age and breed differences in thyroid hormones, insulin-like growth factor (IGF)-I and IGF binding proteins in female horses. J Anim Sci 1996; 74: 1936-1942.

2. Champion ZJ, Breier BH, Ewen WE, Tobin TT, Casey PJ. Blood plasma concentrations of insulin-like growth factor-I (IGF-I) in resting Standardbred horses. Vet J 2002; 163: 45-50.

3. Nelson DL, Cox MM. Lehninger Principles of Biochemistry. 6th ed. Philadelphia, PA, USA: W.H. Freeman; 2013.

4. Jalali M. Thyroid hormones profile following bolus intravenous glucose administration in different physiological states of high producing Holstein dairy cows. MSc, Shiraz University, Shiraz, Iran, 2015.

5. Saadat Akhtar I. Evaluating the relationship among insulin resistance, thyroid hormones and prolactin in different pre and post parturition periods of Iranian fat-tailed sheep. MSc, Shiraz University, Shiraz, Iran, 2016.

6. Henneke DR, Potter GD, Kreider JL, Yeates BF. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet J 1983; 15: 371-372.

7. Huszenicza GY, Nagy P, Juhasz J, Korodi P, Kulcsar M, Reiczigel J, Guillaume D, Rudas P, Solti L. Relationship between thyroid function and seasonal reproductive activity in mares. J Reprod Fertil Suppl 2014; 56: 163-172.

8. Gueorni VH, Bertchinger H. Effect of exposure to a hot-humid and a hot-dry environment on thyroid hormone values in sheep. British Vet J 1983; 139: 119-128.

9. Jenkins TC. Lipid metabolism in the rumen. J Dairy Sci 1993; 76: 3851-3863.

10. Ingraham RH, Stanley RW, Wagner WC. Seasonal effects of tropical climate on shaded and nonshaded cows as measured by rectal temperature, adrenal cortex hormones, thyroid hormone, and milk production. Am J Vet Res 1979; 40: 1792-1797.

11. Mehta V, Sarvaiya N, Pathak M, Patel A. Effect of body cooling during intense summer on circulating thyroid-hormone levels in Surti buffalos. Indian J Anim Sci 1991; 61: 187-188.

12. Mixner JP, Kramer DH, Szabo KT. Effects of breed, stage of lactation, and season of year on thyroid secretion rate of dairy cows as determined by the chemical thyroxine turnover method. J Dairy Sci 1962; 45: 999-1002.

13. Jamshidi A. Evaluation of the relationship between thyroid hormones and lipid metabolism biomarkers in lactation cycles of adult Holstein Cows. MSc, Shiraz University, Shiraz, Iran, 2006.

14. Gooraninejad S, Negahdar MA, Fatemi Tabatabaei SR, Barati F. The effects of age, sex, season and reproductive states on thyroid function of Arab horses in Khuzestan. J Vet Res 2014; 69: 165-172.

15. Maddahi A, Mirzadeh K, Tabatabaei S, Mamouie M, Najafzadeh H. Investigation of the effect of age and season on function of thyroid gland in Arabian male horse. Iran J Anim Sci Res 2016; 26: 63-74.

16. Ghadrdan Mashhadi A, Khadjeh GH, Mohammadzadeh M. The comparison of serum level of T3 and T4 in hot and cold season in Arab horse in Ahvaz. Iranian Journal of Veterinary Medicine 2009; 5: 56-61.

17. Hayirli A. A mathematical model for describing dry matter intake of transition dairy cows. J Anim Sci 1998; 76: 296.

18. Eswari S, Viswanathan S, Leeta V, Nayeem M. Influence of age and sex on thyroxine secretion rate in Madras Red sheep. Indian Vet J 1999; 76: 208-210.

19. Maharajan G, Kumaresan A, Singh A. Serum thyroxine concentrations and free thyopac index in sheep and goats indigenous to Northern Nigeria. Indian J Anim Sci 1982; 52: 1060-1062.

20. Emery RS, Herdt TH. Lipid nutrition. Vet Clin North Am Food Anim Pract 1991; 7: 341-352.

21. Prasad MC. Thyroid activities in sheep during different physiological status. Indian J Anim Sci 1990; 60: 169-171.

22. Ghorbani A, Darmani Kuhi H, Mohit A. Evaluation of blood serum mineral and thyroid hormone concentration of Caspian Miniature horses fed with different diets. Iranian Journal of Veterinary Medicine 2016; 12: 66-77.

23. Nazifi S, Saeb M, Abedi M. Serum lipid profiles and their correlation with thyroid hormones in clinically healthy Turkman horses. Comp Clin Path 2003; 12: 49-52.

24. Frank N, Geor RJ, Bailey SR, Durham AE, Johnson PJ. Equine metabolic syndrome. J Vet Intern Med 2010; 24: 467-475.

25. Christison GI, Johnson HD. Cortisol turnover in heat-stressed cows. J Anim Sci 1972; 35: 1005-1010.

26. Steinhardt M, Thielscher HH. Transport stress in monozygotic twin calves-effects of husbandry systems on metabolic and hormonal variables. Tieraerztl Umschau 2003; 58: 645-650.

27. Aurich J, Wulf M, Ille N, Erber R, Von Lewinski M, Palme R, Aurich C. Effects of season, age, sex, and housing on salivary cortisol concentrations in horses. Domest Anim Endocrinol 2015; 52: 11-16.

28. Hart KA, Wochele DM, Norton NA, Mcfarlane D, Wooldridge AA, Frank N. Effect of age, season, body condition, and endocrine status on serum free cortisol fraction and insulin concentration in horses. J Vet Intern Med 2016; 30: 653-663.

29. Greenspan SL, Rowe JW, Maitland LA, Mcaloon-Dyke M, Elahi D. The pituitary-adrenal glucocorticoid response is altered by gender and disease. J Gerontol 1993; 48: 72-77.

30. Cavinder CA, Vogelsang MM, Gibbs PG, Forrest DW, Schmitz DG. Endocrine profile comparisons of fat versus moderately conditioned mares following parturition. J Equine Vet Sci 2007; 27: 72-79.

31. Macpherson ML, Simmen RCM, Simmen FA, Hernandez J, Sheerin BR, Varner, DD, Rigby S. Insulin-like growth factor-I and insulin-like growth factor binding protein-2 and-5 in equine seminal plasma: association with sperm characteristics and fertility. Biol Reprod 2002; 67: 648-654.

32. Noble GK, Houghton E, Roberts CJ, Faustino-Kemp J, De Kock SS, Swanepoel BC, Sillence MN. Effect of exercise, training, circadian rhythm, age, and sex on insulin-like growth factor-1 in the horse. J Anim Sci 2007; 85: 163-171.

33. Fortier LA, Kornatowski MA, Mohammed HO, Jordan MT, O'cain LC, Stevens WB. Age-related changes in serum insulin-like growth factor-I, insulin-like growth factor-I binding protein-3 and articular cartilage structure in Thoroughbred horses. Equine Vet J 2005; 37: 37-42.

34. Corpas E, Harman SM, Blackman MR. Human growth hormone and human aging. Endocr Rev 1993; 14: 20-39.

35. Carlson HE, Gillin JC, Gorden P, Snyder F. Absence of sleep-related growth hormone peaks in aged normal subjects and in acromegaly. J Clin Endocrinol Metab 1972; 34: 1102-1105.

36. Clapper JA, Clark TM, Rempel LA. Serum concentrations of IGF-I, estradiol-17β, testosterone, and relative amounts of IGF binding proteins (IGFBP) in growing boars, barrows, and gilts. J Anim Sci 2000; 78: 2581-2588.

37. Sticker LS, Thompson DL, Fernandez JM, Bunting LD, DePew CL. Dietary protein and (or) energy restriction in mares: plasma growth hormone, IGF-I, prolactin, cortisol, and thyroid hormone responses to feeding, glucose, and epinephrine. J Anim Sci 1995; 73: 1424-1432.