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Seasonal changes in serum progesterone levels in Caspian mares

Reza Haghdoost[a], Mohammad Roostaei-Ali Mehr[a], Ahmad Ghorbani[b]

[a] Department of Animal Science, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.
[b] Animal Science Research Department, Guilan Agricultural and Natural Resources Research and Education Center, AREEO, Rasht, Iran.

ABSTRACT

The present study was conducted to assess the seasonal breeding of Caspian horses in 10 mares during a year. Mares were divided into two age groups: 3-6 years (young) and 7-19 years (old). Blood samples (n=530) were collected weekly. The ovarian activity was evaluated by the concentration of progesterone. Mares with serum progesterone concentrations consistently higher and lower than 1 ng/ml were considered cyclic and non-cyclic, respectively. Results showed an interaction between time and age on the concentration of serum progesterone, ovarian activity, body weight, and body condition score (BCS) ($p < 0.05$). In March, the concentration of serum progesterone was higher in young mares (7.84 ± 1.14) than in old mares (1.26 ± 1.14, $p < 0.05$). The serum progesterone was higher in old mares than in young mares during July-November ($p < 0.05$). Ovarian activity was higher in young mares than in old mares during February-April ($p < 0.05$). Ovarian activity was higher in old mares than in young mares during July-November ($p < 0.05$). The length of the breeding season was higher in old mares than in young mares ($p < 0.05$). BCS was higher in young mares (4.4 ± 0.22) than in old mares (3.2 ± 0.22) in February ($p < 0.05$). Body weight was lowest in the young mares during September-January ($p < 0.05$). There was a significant correlation between ovarian activity and BCS of Caspian mares. Finally, seasonal breeding was shorter and earlier in young Caspian mares compared to old mares.

Keywords

Caspian horse, season, breeding, progesterone

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Abbreviations

BCS: Body condition score
ELISA: Enzyme-linked immunosorbent assay

Corresponding author:
Mohammad Roostaei-Ali Mehr
Email: roostaei@guilan.ac.ir
Tel: +98(91)1135-5262

Introduction

The Caspian horse is intelligent and has great potential for training. This animal is very suitable for the equestrian education of children and adolescents due to its gentle behavior and body size. The Caspian horse is an ancient breed of small horse native to northern Iran (around the Caspian Sea) which is reported to be in danger of extinction in its original homeland [1]. Iranian Caspian horses were identified by Louise Firouz in 1966 and these animals were collected by Agricultural Research Education and Extension Organization (AREEO; Iran, Tehran) to be supported in 2008. A herd of 75 heads was provided, but over time, their number decreased. At present, this herd consists of 45 horses. It seems that less than 400 of these horses could be found in Iran [2]. Therefore, it is important to study the reproductive activity of this animal.

In seasonal breeding animals, the function of the reproductive system is adjusted to prevent the born of neonatal during bad weather. The mare is a seasonal polyestrous whose reproductive activity is induced by altering the photoperiod [3]. In addition to photoperiod, nutrition, body condition score (BCS) and environmental temperature affect seasonal reproductive activity in mare [4]. There is an interaction between these factors and photoperiod on the precise onset and duration of ovarian inactivity in the mare [5]. Moreover, the onset and termination of the breeding season may occur independently of a change in photoperiod [3].

Persistent ovarian activity is different in old and young mares [6]. Furthermore, the breed effect was reported for the time of the first ovulation and the end of winter ovarian inactivity in mares [7]. Evidence shows that the mortality rate is higher in foals born at the beginning of the year than in foals born later [8]. Different experimental approaches indicated that the annual reproductive rhythm of the mare, similar to other seasonal animal breeders, has a strong endogenous component [9]. Breeding information is available about some horse breeds. However, seasonal breeding information about Caspian horses is important and these data are needed to support this animal. Therefore, the present study aimed to determine the onset, termination, and length of seasonal breeding in Caspian mares.

Result

The results of analytical validation showed that the serial dilution of the serum Caspian mare was significantly decreased following the dilution level (r^2 = 0.9123, Figure 1). Moreover, the slope of the serial dilution of serum progesterone was also parallel to the respective standard curves ($p < 0.05$). These results indicate that progesterone antigen in Caspian mare serum can bind correctly to the antibody of this assay. The scatter of serum progesterone concentrations in horses during the evaluation is shown in Figure 2. The serum progesterone concentration was higher in old mares than in young mares, (Table 1, p <0.05). The serum concentration of progesterone was higher in spring than in winter (p <0.05). There was an interaction between time and age on the serum concentrations of progesterone, ovarian activity, body weight and body condition score (BCS) (Figure 3, p <0.05).

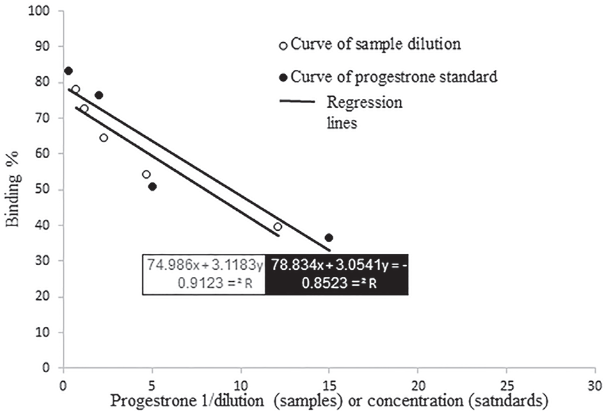

Figure 1.
Serial dilution results of Caspian mare serum are presented. The sample (open symbol) was diluted 1:2, 1:4, 1:8 and 1:16 in assay buffer and tested for binding to the progesterone conjugate antibody in parallel with serially diluted standard (closed symbol).

There was no difference between mares on the serum progesterone from Dec to Feb (Figure 3A, p > 0.05). In March, the concentration of the serum progesterone was higher in the young mares (7.84 ± 1.14) than old mares (1.26 ± 1.14, p <0.05). There was no difference between the serum progesterone of old mares in April (6.36 ±1.14) and the serum progesterone of young mares in March (7.84 ± 1.01, p <0.05). In young mares, the serum progesterone was higher from March to July than that in other months (p <0.05). The serum progesterone of old mares was higher from April to November than that in other months (p <0.05). The serum progesterone was higher in old mares than that in young mares from July to November (p <0.05). In December and January, the minimal concentration of the serum progesterone was observed in all mares.

In young mares, the maximum (>80%) and minimum (<5%) ovary activity were observed from April to January (4 months) and from September to January (5 months), respectively (Figure 3B, p <0.05). In old mares, the maximum (>70%) and minimum (zero) ovary activity were observed from May to October (6 months) and from December to March (3 months),

Table 1.
The main effect of age and time on the serum progesterone, ovarian activity (monthly), body condition score and body weight of Caspian mares

	Variable		Serum progesterone (ng/ml)	Ovarian activity (%)	Body condition score*	Weight (kg)
Age	Young		$3.88^{b} \pm 0.30$	$41.66^{b} \pm 4.53$	$4.40^{b} \pm 0.11$	$191.45^{b} \pm 3.59$
	old		$5.94^{a} \pm 0.30$	$53.41^{a} \pm 4.53$	$4.83^{a} \pm 0.11$	$218.99^{a} \pm 3.59$
Time	Winter	Jan	$0.08^{d} \pm 0.80$	$0.00^{f} \pm 7.4$	$3.20^{e} \pm 0.15$	$198.35^{d} \pm 2.72$
		Feb	$1.58^{d} \pm 0.71$	$14.00^{f} \pm 7.4$	$3.80^{e} \pm 0.15$	$201.20^{c} \pm 2.72$
		Mar	$4.55^{c} \pm 0.81$	$45.00^{e} \pm 7.4$	$4.6^{c} \pm 0.15$	$204.55^{c} \pm 2.72$
	Spring	Apr	$7.3^{b} \pm 0.71$	$80.00^{b} \pm 7.4$	$5.40^{b} \pm 0.15$	$208.50^{b} \pm 2.72$
		May	$9.85^{a} \pm 0.80$	$100.00^{a} \pm 7.4$	$5.90^{b} \pm 0.15$	$211.60^{a} \pm 2.72$
		Jun	$9.43^{a} \pm 0.71$	$98.00^{a} \pm 7.4$	$6.30^{a} \pm 0.15$	$213.80^{a} \pm 2.72$
	Summer	Jul	$9.30^{a} \pm 0.80$	$80.00^{b} \pm 7.4$	$5.50^{b} \pm 0.15$	$210.05^{ab} \pm 2.72$
		Aug	$7.20^{b} \pm 0.71$	$66.00^{c} \pm 7.4$	$5.20^{b} \pm 0.15$	$207.45^{b} \pm 2.72$
		Sep	$3.70^{cd} \pm 0.80$	$42.00^{d} \pm 7.4$	$4.20^{c} \pm 0.15$	$205.95b^{c} \pm 2.72$
	Fall	Oct	$4.04^{c} \pm 0.80$	$35.00^{e} \pm 7.4$	$4.10^{c} \pm 0.15$	$202.35^{c} \pm 2.72$
		Nov	$1.66^{d} \pm 0.80$	$10.00^{f} \pm 7.4$	$3.60^{e} \pm 0.15$	$200.50^{c} \pm 2.72$
		Dec	$0.13^{d} \pm 0.80$	$00.00^{f} \pm 7.4$	$3.20^{e} \pm 0.15$	$198.45^{d} \pm 2.72$

$^{a\text{-}g}$ different superscripts denote significant differences ($p < 0.05$)

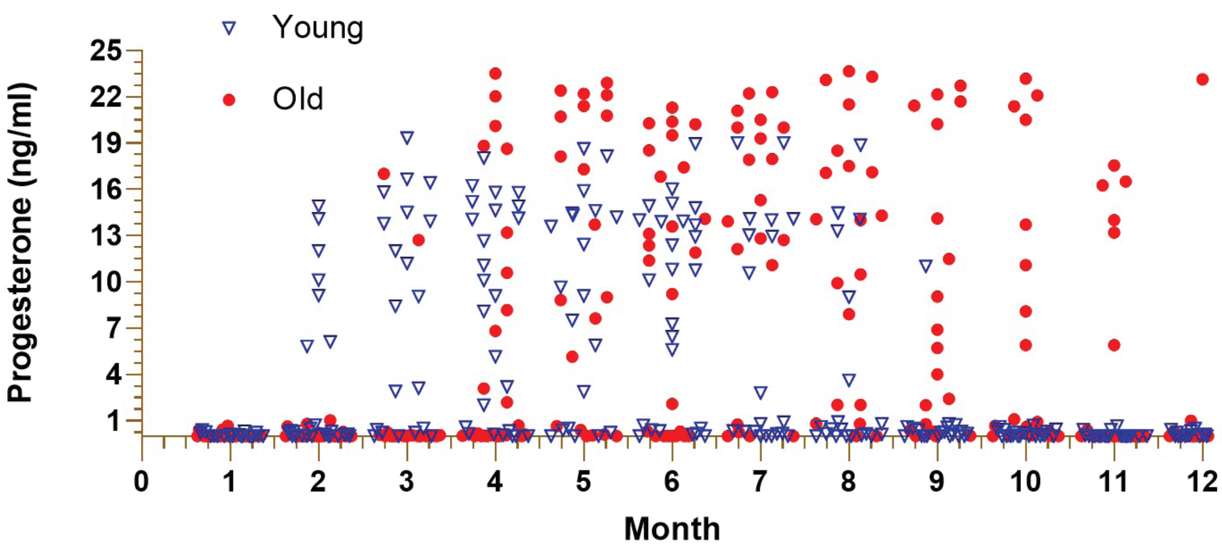

Figure 2.
Weekly changes in the serum progesterone concentration of each mare throughout the year

respectively (p <0.05). Ovary activity was higher in young mares than old mares from February to April (p <0.05). Ovary activity was higher in old mares than young mares from July to November (p <0.05). In May, ovary activity was highest in all mares (p <0.05). The length of the breeding season was higher in old mares than that in young mares (Table 2, p <0.05).

In December and January, the body condition score was minimum in all mares and there was no difference between mares (Figure 3C, p > 0.05). The body condition score was higher in young mares (4.4 ± 0.22) than that in old mares (3.2 ± 0.22) in February (p <0.05). In October and November, the body condition score was higher in old mares (p <0.05). Body weight was lower in the young mares (Figure 3D, p

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<0.05). In January and July body weight was highest in old mares (p >0.05). Body weight was lowest in the young mares from September to January (p <0.05). There was a significant correlation between ovarian activity and body condition of Caspian mares (Table 3).

Table 2.
The length of the breeding season in Caspian horses

Age	The length of the breeding season (week)	From the winter solstice to the beginning of the breeding season (week)*	From summer revolution to the end of the breeding season (week)
Young	$23.00^{b} \pm 1.63$	$13.00^{b} \pm 1.15$	$10.00^{b} \pm 2.09$
Old	$27.80^{a} \pm 1.63$	$19.40^{a} \pm 1.15$	$21.40^{a} \pm 2.09$

a-c different superscripts denote significant difference (p < 0.05)

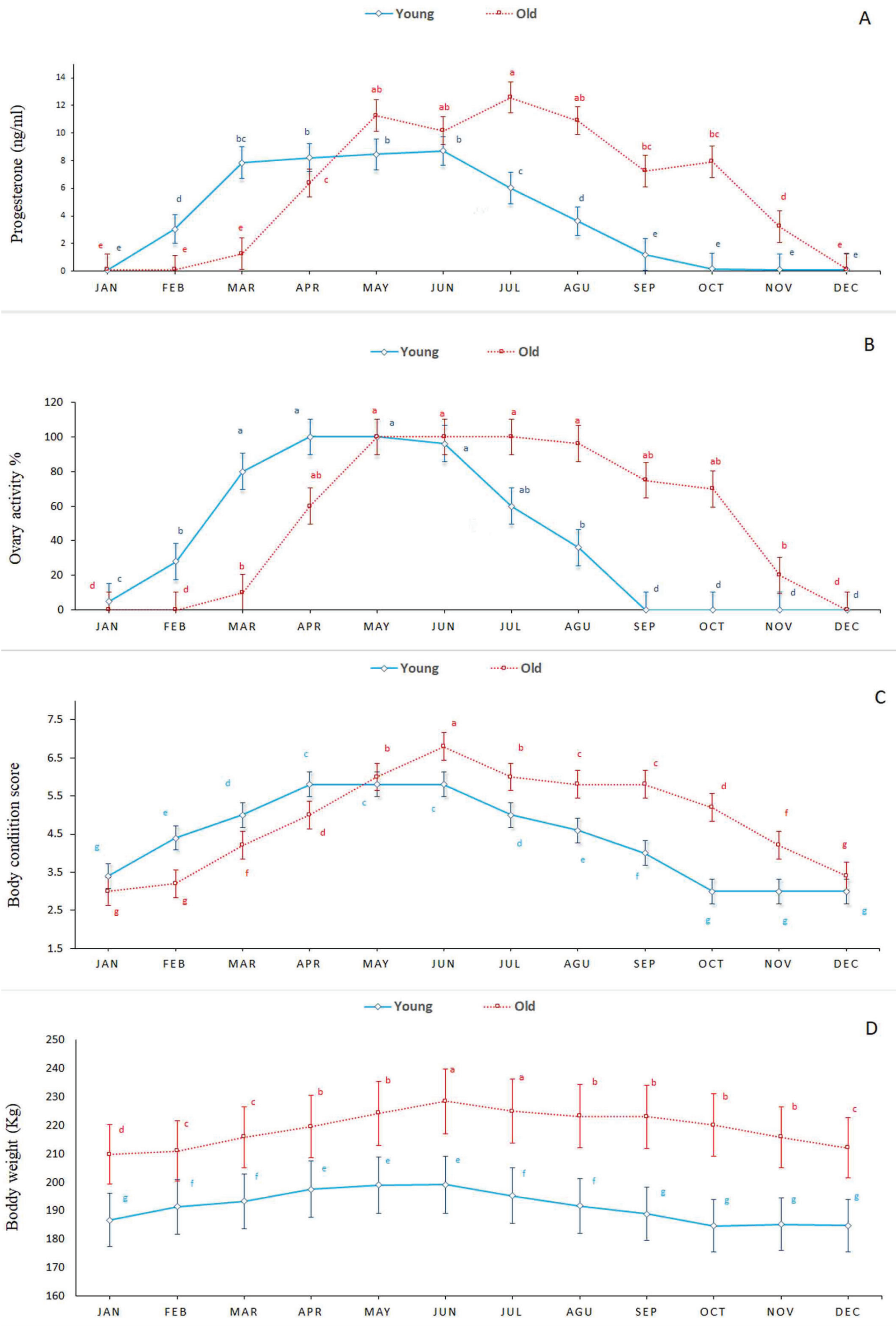

Figure 3.
The interaction effect of age and time on serum progesterone concentration (A), ovarian activity (B), BCS (C) and weight (D) in Caspian mares. a-g different superscripts denote significant differences (p < 0.05).

Table 3.
Correlation between ovarian activity, body condition and body weight in Caspian mares

Variable	Bodyweight (Kg)	Body condition	Ovarian activity
Ovarian activity	0.45256	0.91434	1.0000
	< 0.0001	< 0.0001	
Body condition	0.52675	1.0000	
	< 0.0001		
Body weight	1.0000		

Discussion

The results of the current research showed that the progesterone concentrations of young and old mares gradually began to increase at the beginning of the breeding season in February. Moreover, progesterone concentration was a lot higher in young mares than in old mares in March. Consequently, the spring transition in young mares started sooner than in old mares. In equine, a relative deficiency in the steroidogenic enzymes P450scc, P450c17, and P450arom was reported in spring transition [10]. It was mentioned that deficiency in steroidogenesis is due to the lack of gonadotrophic support caused by the low circulating concentrations of LH and low levels of gonadotropin receptors during the spring transition [11]. Supplementation of transitional mares with gonadotrophins in the form of hCG stimulates steroidogenesis [12]. Therefore, it seems that the ovarian steroidogenesis of young Caspian mares resumed earlier than old mares in the spring transition.

The BCS of young mares (> 4) was higher than old mares at the beginning of the breeding season (in February). Moreover, the spring transition and seasonal breeding of young mares began earlier than old mares. There was a significant correlation between ovarian activity and BCS, which is in line with the findings of Vecchi et al. [13]. Consequently, the onset of the breeding season might be modulated by the percentage of body fat and BCS in the Caspian mare. It has been mentioned that reproductive function is strongly influenced by metabolic hormones in mares [14]. Insulin can cross the blood-brain barrier and insulin receptors have been observed in several brain areas, including the hypothalamus [15]. Increased adiposity leads to a higher circulating concentration of insulin [16]. Moreover, hyperinsulinemia was observed in young mares in late winter [17]. Throughout the year, there is a correlation (0.64, $p < 0.001$) between insulin concentration and fat thickness in the mare [5]. Insulin modulates GnRH secretion [18, 19] and it seems to be also important for LH release [20]. However, in the equine species, this effect is controversial [21]. Findings suggest that the BCS and adiposity of Caspian mares are very important at the beginning of the breeding season.

This is the first study to report seasonal luteal activity in Caspian mares and a high percentage of horses with luteal activity in late spring and early summer. At the end of the breeding season, it was observed that the fall transition of young mares was during August-October. However, the fall transition of old mares was during late October-December. The breeding season of old mares started late but it took longer than young mares, which is similar to the results of other researchers [17, 22]. On the other hand, the BCS of old Caspian mares was > 4 from May to the end of November. It has been shown that the ovarian activity of the mares with high BCS continues during the winter conversely mares with BCS of < 3.5 [6]. During three years, BCS had a strong correlation with ovarian activity in Welsh ponies [5]. However, the ovarian activity of old Caspian mares, the same as the young ones, was completely consistent with the changes in the BCS throughout the year.

Our results showed differences between young and old mares regarding progesterone concentration, luteal activity, and BCS during the year. It was demonstrated that the concentration of serum leptin augmented with age and there was a positive correlation between BCS and serum leptin in horses, as leptin rose to 1.11 ± 0.57 ng/mL for one score increase in BCS [23]. Leptin, a hormone predominantly released by adipose cells, helps to regulate energy balance by inhibiting appetite [24]. On the other hand, low metabolic rates during winter (declining during autumn to reach a minimum during December and January) and the resumption of high metabolic activity in spring (augmenting exponentially during March and April to the annual peak in May) are well known in horses [25]. Therefore, the observed difference between young and old mares may have been due to seasonal changes in the metabolic rate and secretion of adipokines, such as leptin.

In conclusion, there were differences between young and old Caspian mares in the onset, termination, and length of the seasonal breeding of mare Caspian. Compared to old Caspian mares, the ovarian activity of young mares started and ended earlier. The length of the breeding season of young Caspian mares was shorter, whereas the ovarian activity of old Caspian mares continued until midwinter. There was a significant correlation between progesterone concentration and ovarian activity. Therefore, the differences observed between the age groups of Caspian mares at the onset and end of the breeding season may result from the changes in BCS. It seems that age per se may not be an important variable.

Materials and Methods

Subjects and sample collection

The experiment was conducted on ten Caspian mares of the experimental herd from the Iran Meteorological Organization during June 2018-July 2019. Experimental procedures and protocols were performed under National Animal Ethics approved by the Veterinary Organization of Iran. Mares had not nursed a foal the previous year. These ten mares were randomly selected and divided into two age groups: 3-6 years (young) and 7-19 years (old). Mares had not nursed a foal the previous year. The horses were fed a diet formulated based on the National Research Council [25] feeding standard, body weight, age, and physical activity. The total daily dry matter intake of the horses was 2%-2.5% of body weight, with the same amount of forage and concentrate. The animals had free access to water and a salt mineral lick. The horses were given one-half of their daily diet of alfalfa hay and concentrate (Table 4) at 08:00 and 10:00, and the rest at 16:00 and 18:00, respectively. Housing and management conditions were the same for all animals. During the night, mares were housed in covered stalls and during the day they had access to a 300 m2 paddock.

Normal husbandry procedures, worm treatment, and vaccination programs were followed. All mares were kept under natural photoperiod, which at this latitude (37° 12´north latitude and 49° 39´east longitude) ranges from 8 h of light at the winter solstice to 16 h of light at the summer solstice. BCS and weight of mares were defined monthly [26, 27].

Blood samples

Since the beginning of the experiment, blood samples were collected by venipuncture of the jugular vein weekly. After clotting, sera were separated by centrifugation and were immediately used to determine progesterone concentration. The ovulatory activity was evaluated based on the concentration of progesterone.

ELISA

Serum progesterone was assessed in duplicate using a commercial ELISA kit (Monoblind, USA). A parallelism test was performed to define the reliably measure progesterone concentrations in the equine using a commercial ELISA. To examine the capability of commercial progesterone ELISA kits for measuring progesterone in Caspian horses, analytical validation was performed. The analytical validation comprises of parallelism test. Briefly, two sera from Caspian mares were diluted (1:2 to 1:16) using assay buffer. Diluted serum was then assayed together with progesterone standard (serial dilution of progesterone standard was 0.3-15 ng/ml). Afterward, the test of the equality of slope was performed following Zar [28] to compare the slope of the expected dose versus the percent bound of diluted serum with the slope of the standard dilutions.

Serum progesterone concentration

Mares were considered cyclic if serum progesterone concentration changed from < 1 ng/ml to > 1 ng/ml during 4 weeks. They were classified as being in anestrus if their progesterone levels were < 1 ng/ml for more than 4 consecutive weeks. The last date when the progesterone concentration was > 1 ng/ml in the last ovulatory cycle of the breeding season was deemed as the start of the anovulatory period, and the first date when progesterone was > 1 ng/ml in the first ovulatory cycle of the new breeding season was deemed as the end of the anovulatory period. Ovarian activity per month was calculated based on the following equation:

(Total number of samples per month-Number of samples (per month) indicated the anovulatory cycle)/(Total number of samples per month)×100

The relationships between ovarian activity, weight, BCS, and time were evaluated by the linear correlation analysis (CORR procedure) using SAS [29]. The data regarding serum progesterone concentration were analyzed by the MIXED procedure of SAS with repeated measures data, and least-squares means were compared using Tukey's adjusted method. The data on ovarian activity, weight, and BCS were analyzed by completely randomized design in a 3 (age groups) × 12 (months) factorial arrangement of 36 treatment combinations as fixed effects and time as a repeated measure. Mare was considered a subject in this experiment. The data on the length on the breeding season, the time interval between the winter solstice and the beginning of the breeding season, and the time interval between the summer solstice and the end of the breeding season were analyzed by a completely randomized design. Means were compared using Tukey's test and differences were considered to be statistically significant at $p < 0.05$.

Table 4.
Composition of feeds used for experimental mares.

Nutrients	Commercial concentrate	Alfalfa Hay
DE	3.4 Mcal/kg	2.43 Mcal/kg
CP	140 g/kg	150 g/kg
Ca	7.95 g/kg	14.7 g/kg
P	7.5 g/kg	2.8 g/kg
Mg	1.75 g/kg	2.9 g/kg

DE = Digestible Energy, CP = Crude Protein

Authors' Contributions

MRAM designed the experiment. RH and AG collected the samples. RH performed laboratory measurements. MRAM performed the analysis of data and wrote the manuscript. All authors contributed to data interpretation and revising the manuscript, as well as read and approved the final manuscript. MRAM had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Competing Interests

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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