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Role of Leptin and Insulin like Growth Factor-I in Breeding Egyptian Horses along the Year

Amal M. Abo El-Maaty[1], Mohamed S. Kotp[1], Magda M. El-Tohamy[1], Walid S. EL Natat[1] and Amira H. Mohamed[2]

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[1]Animal Reproduction and AI Dept., Veterinary Division, National Research Center, Dokki, Egypt
[2]Department of Clinical Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt.

ABSTRACT

To confirm that mares are regular polyestrous animal all the year round, ovarian follicles, corpora lutae were detected with ultrasound and blood samples were collected with all ultrasonographic examination of 516 non pregnant mares. Blood samples were assayed for progesterone, leptin and Insulin like growth factor –I (IGF-1) using commercial ELISA kits. Progesterone levels > 2 ng/mL accompanied a corpus luteum on ovary all months of the year and progesterone levels <1ng/ml accompanied a large follicle >30 mm in diameter in addition to estrous behavior indicated regulatory of estrus. As well as, commencement of foaling from December to May (Winter and Spring) each year indicate that horses are not a long day breeder and that horse breeders prefer Winter and Spring to breed their mares due to the suitable environmental temperature and the availability of good quality green clover to feed their mares after expected foaling within the same seasons. Leptin levels decreased significantly during September while IGF-1 levels decreased significantly during June and July. In conclusion, mares of Egypt cycle all the year round but breeding them depends on their breeder decision. Leptin and IGF-1 have no role in breeding horses in Egypt.

Key words: Oestrus Cyclisity, Leptin, Progesterone, IGF-1 and Mare.

*Corresponding author. E-mail: amalaboelmaaty1@yahoo.com.

INTRODUCTION

In many countries where spring and summer are characterized by long photoperiod, mare is normally a seasonal breeder with cyclic activity occurring from spring to autumn; during the winter mares will normally become anesterus and the winter anoesterus is followed by a period of transition to regulate cyclic activity (Arthur et al., 1989). Ovarian activity increased by increasing the day light length in the temperate latitudes (Greenhoff and Kenney, 1975; Nequin et al., 2000). Higher frequency of estrus were observed during the spring and summer (the breeding season) than during the autumn and winter (anestrous season, Daels and Hughes, 1993). However, mare's exhibited reproductive activity during the non-breeding season (Ginther, 1974) were having higher plasma concentration of leptin, body weight and estimated percent of body fat (Fitzgerald and McManus, 2000). Some reports mentioned that light horse breeds maintain regular estrous cycles throughout the winter rather than transitioning into anestrus (King et al., 1988) and others reported that photoperiod has no role in breeding mares (Colquhoun et al., 1987).

Previous reports of Egypt observed that, native Egyptian mares including Arabians, estrus occurs in mares all round the year with less frequency during the summer and autumn than the winter and spring (El-Wishy et al., 1990; Abu-Atiah, 1997). Several studies suggest that leptin modulates both reproductive and immune functions (Drazen et al., 2000, 2001).

In Lusitano mares, increased leptin concentrations might be involved in the stimulation of the hypothalamus–pituitary–gonad function during long days. Increased concentrations of this hormone in circulation might be associated with the restart or maintenance of ovarian cyclisty (Ferreira-Dias et al., 2005). Response of the ovaries to endocrine stimuli during the anovulatory season is affected not only by circulating concentrations of trophic hormones but also by locally produced growth factors (oestradiol, IGF-1, IGFBPs and VEGF) that are putative modulators of follicular responses to gonadotropins (Donadeu and Watson, 2007). Within our locality, reports concerning seasonal breeding of mares are controversy but does native Arabian horses or imported breeds from colder countries with marked seasonal difference in photoperiod, inhabited for several years in Egypt, exhibit seasonal breeding pattern, this what this study will explore.

Therefore, this study aimed to determine ovarian cyclisity all the year by monitoring estrus behavior, scanning ovaries by ultrasound to monitor ovulating follicle and/or developing corpora lutae and measuring progesterone in non pregnant mares and foal crop during one year in pure Arabian and imported European breeds and to distinguish the role of leptin, and IGF-1 in breeding mares.

MATERIALS AND METHODS

Animals

Brood mares (4 to 15 years old, n=516) belonged to El Basaten horse club, Police Academy horse stud (Cairo, Ministry of Interior), Al Zahraa Pure Arabian Horse Stud, Private Arabian horse studs and Imported European Horse belonged to several Horse clubs (Cairo). Arab mares (n=421) and Foreign mares (n=92) were kept in an indoor paddock with partition individually. Mares were kept under natural day light and temperature. Mares from several horse studs were maintained on commercial pelleted ration and hay with free access to water, barley and hay during summer and barley and green quality Egyptian clover green quality (*Trifolium alexandrinum*) from October to May every year from 2011 to 2013.

Ultrasound Scanning

A multi-frequency 2.6-7.5 MHz endorectal transducer of NOVEK ultrasound scanner (Germany) belonged to Police Academy horse farm and a scanner 200 (Pie medical, Netherlands) equipped with 6 to 8 linear-array real time B-mode transducer were used for examining mares at weekly intervals before breeding for detection of mature Graffian follicle (≥3 cm) as described previously (Ginther, 1995). When a large follicle >30 mm and uterine edema were scanned, mare was referred to a stallion for confirming estrus signs and excluding persistent non-ovulatory follicle.

Blood Sampling and Hormonal Assaying

Blood samples were collected via Jugular vein puncture in plain vacuum tubes from non pregnant animals only. Sera was harvested and stored at -20°C till hormone assaying. Serum progesterone was analyzed using a commercially available Enzyme immunoassay kit supplied by Medical Biological Service S.R.L. (Milano, Italy). Sensitivity of the assay was 0.1ng/ml and intra- and inter-assay CV was 10.6 and 12.6%. Leptin was assayed using Leptin ELISA (Sandwich) previously used to horses in our Laboratory (Abo El-Maaty and Gabr, 2010) and IGF-1 was assayed (Abo El-Maaty et al., 2013) using DRG diagnostics (DRG, Germany). Sensitivity of the assay, intra- and inter-assay coefficients was 1.29 ng/ml, 6.62 and 7.79% for IGF-I and 1.0 ng/ml, 3.1 and 9.7% for leptin.

Statistical Analysis

Data are presented as Mean± SEM (Standard error) using SPSS software (SPSS, 2007). Analysis of variance simple one way ANOVA was used to compare between levels of progesterone, Leptin and IGF-1 during months of the year for all animals and each breed studied. Results are presented in plots with error bars.

RESULTS

It is obvious from (Figure 1) that mean progesterone concentrations >2 ng/ml indicates ovulation all months of the year. Significant (P=0.0001) higher progesterone concentrations are obtained in June and July but significant low mean progesterone concentration was observed in February. Presence of progesterone levels

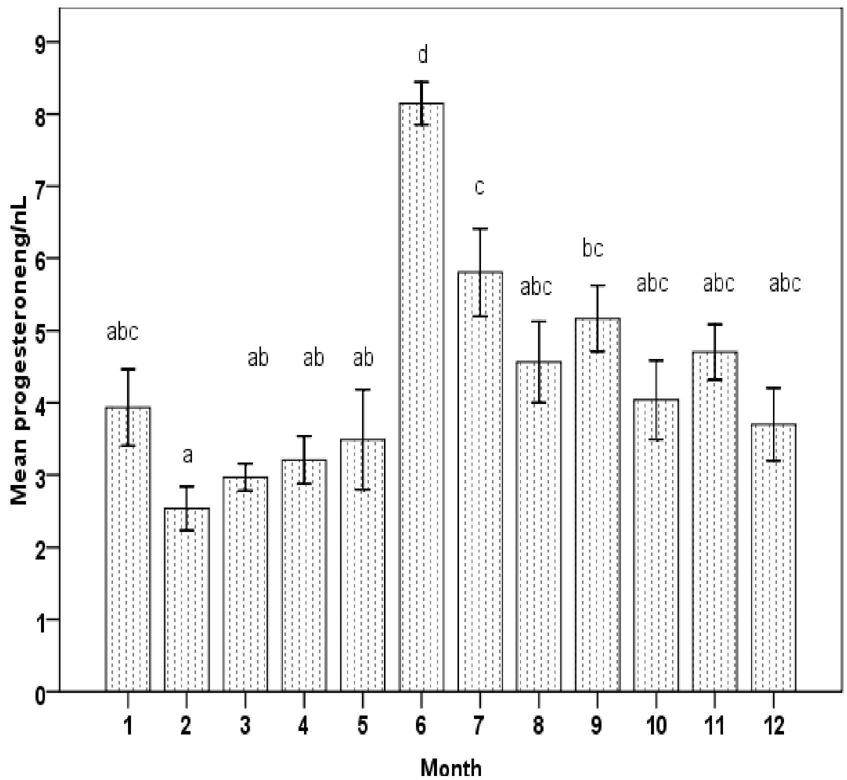

Figure 1. Mean progesterone concentrations during different months of the year. Different letters a, b, c and d indicates significance at P<0.05.

<1ng accompanied large perovulatory follicle were also noted all the year round (Figure 2). From the foaling records of one breeding season of one farm, births commences from December to June indicating ovulations from November to July of the previous breeding season (Table 1). Mean progesterone levels ≥1.5ng/mL are obtained in both native Arabians and imported breeds of mares during all months of the year (Figure 3), indicating no intensity in estrous whatever the season of the year and breeding a mare is her breeder decision not her seasonality in breeding. In native Arabian mares, significant (P=0.0001) lowest mean progesterone levels are observed in May but P_4 levels are significantly highest from June to November. Also, imported mares have significant (P=0.002) low mean progesterone levels during October and from February to March but significant high levels are observed in July. Significantly (P=0.0001) low mean leptin hormone concentrations are observed during July and September and significantly high mean level was observed during March (Figure 4).

Significant high leptin levels are observed from January to June and from October to December. Low leptin concentrations are observed in both Arab and foreign mares from June to November (Figure 5) while significant high leptin levels are observed in Arab mares from December to April and in foreign breeds of mares from January to May (Figure 5). Insulin like growth Factor-I (IGF-1) levels are significantly (P=0.0001) low during June and July (Figure 6 and 7) compared to the other months of the year. The same significant (P=0.0001) decrease of IGF-1 concentrations was also observed during June and July in both breeds studied (Figure 7).

DISCUSSION

Outcomes of breeding mares and hormones related to ovulation (progesterone), body condition (leptin) and metabolic status (IGF-1) all indicated absence of seasonal breeding in mares of our latitude. Previous studies on mares proved breeding and foal crop during winter (Abo El-Maaty and Gabr, 2010) and estrous and ovulation was also studied and recorded during summer (Abo El-Maaty and El-Shahat, 2012). Although our

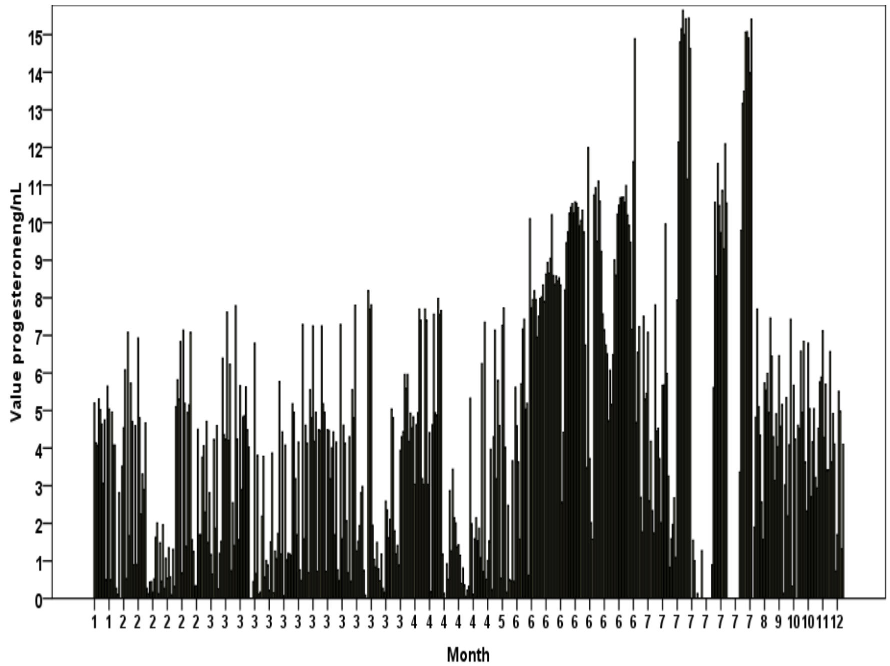

Figure 2. Progesterone (ng/mL) levels of individual mares during different months of the year.

Table 1. Number of mares exhibiting foal heat, number of covers, average inseminations, pregnancy rate, from December to the next May in one breeding season.

Month	Foaling mares	Total covers	Covers /mare	Estrous days	Estrous phase	Foal heat Pregnancy rate
December	50	103	2.06	250	5.2	30
January	27	75	2.78	164	5.8	15
Feb	32	85	2.66	190	5.9	20
Mar	46	78	1.69	245	5.3	26
April	32	72	2.25	172	5.3	19
May	35	86	2.46	197	5.6	15
June	23	55	2.39	142	6.1	14
Total	245	554	16.29	1360	39.2	139
Mean	35±3.69	79.14±5.56	2.33±0.14	194.3±15.3	5.6±0.13	19.86±2.30

country belongs to the north hemisphere, but it seems that the photoperiod has no effect on breeding mares of our latitude and its effect appear where there is a wide difference in photoperiod between seasons of the year as countries away from the equator became colder and has no effect as countries near the equator and became warmer. In such colder countries away from the equator, the horse is a seasonal polyestrous species with onset of the breeding season occurring in spring and is associated with increase in daylight, temperature and availability of food. Breeding season occurs from April to September in the Northern Hemisphere (Hughes et al., 1975). Other studies reported that the onset and termination of the breeding season closely coincide with onset and termination of the pasture season (Ginther, 1974).

In horses and other species, seasonal reproductive inactivity may be the result of a direct reactive inhibition induced by signals such as short day length, adverse

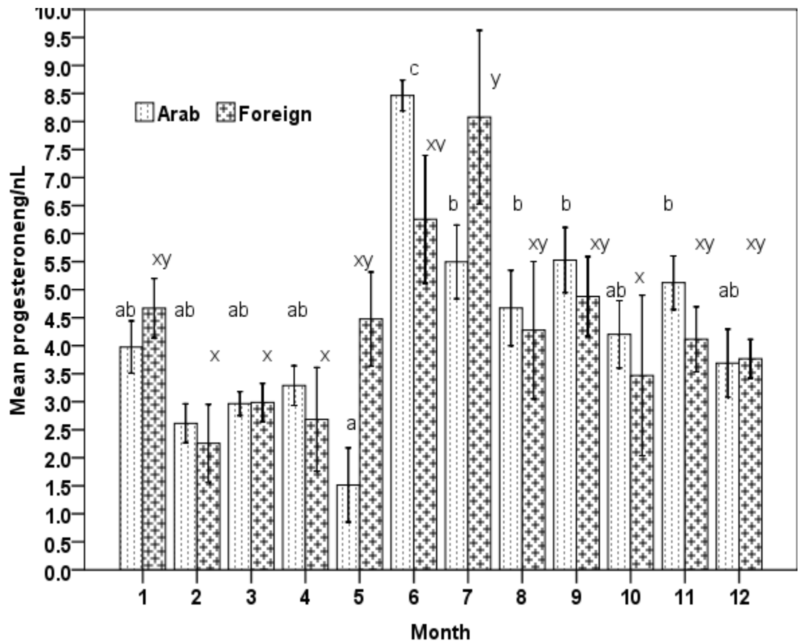

Figure 3. Mean progesterone concentrations during months of the year in native Arab and Foreign imported mares. The letters a, b and c indicates significance of Arab mares and the letters x and y of Foreign mares.

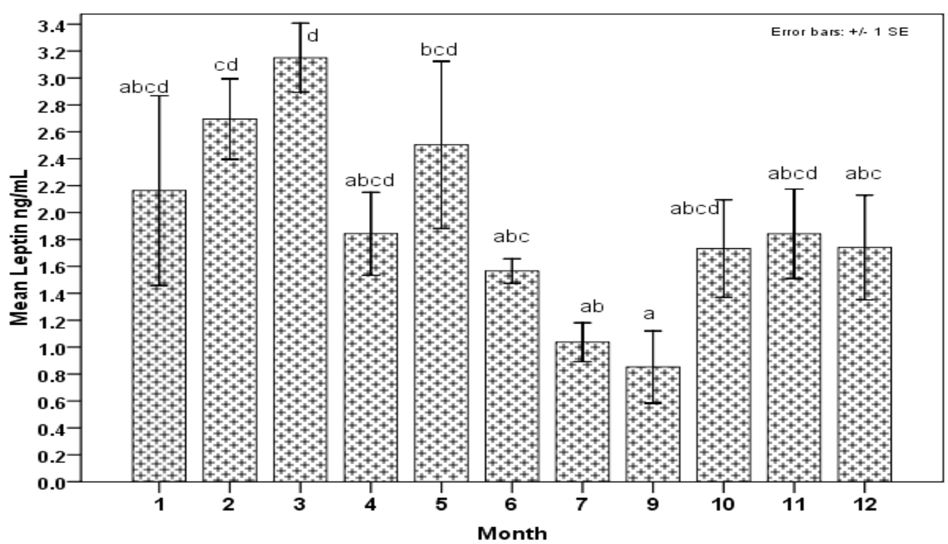

Figure 4. Mean levels of leptin hormone in mares (ng/mL) during different months of the year. Different letters a, b, c and d indicates significance at P<0.05.

climate, poor nutrition (Nagy et al., 2000). Recent studies also confirmed this present findings, the mare is seasonally polyestrus, having an a non-ovulatory period during the short light days of late fall and early winter, and beginning to ovulate as the days become longer during the winter (Crowell-Davis, 2007). In mares with distinct anestrum season, short day length was associated with a decrease in gonadotropin secretion and consequently a decrease in ovarian activity and the absence of cyclic activity was referred to a lack of

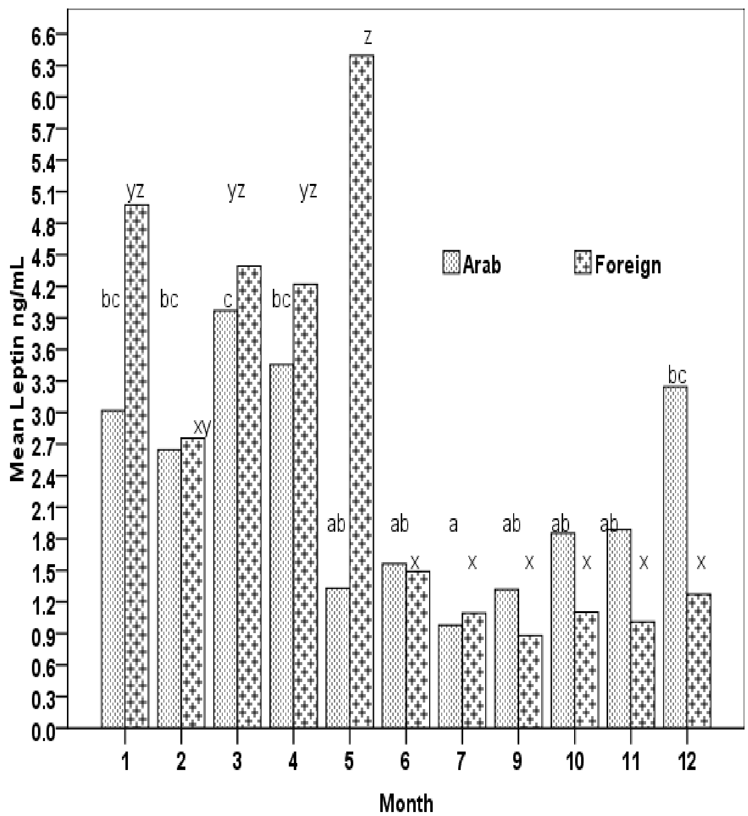

Figure 5. Mean leptin levels in Arab and Foreign imported mares during different months of the year. Letters a, b and c indicates significance of Arab mares and x, y and z of foreign mares.

positive signals for example, long day length, favorable climatic and nutritional conditions, the presence of a stallion, that stimulate GnRH and gonadotropin secretion during the breeding season. Seasonal changes in luteolytic function are caused by an alteration in the signal for PGF2 release (King et al., 2010). Contrary to the previous reports, horses of south Mediterranean are polyestrous animals that cycle all the year round. Although, most Arab countries belong to Northern Hemisphere, the seasonal difference of day light is not as obvious as North Mediterranean countries. Results of progesterone and foaling records confirmed that Arab mares cycle all the year round but breeding them coincide with onset and termination of the good quality green clover which extends from October to May. Even more, imported horses- from colder countries with marked seasonal difference in day length, attain the similar pattern of breeding and ovulate all the year similar to Arab horses. As well as, Crowell-Davis (2007) referred the presence of mares exhibiting estrous behavior periodically during a non-ovulatory period to the release of estrogenic steroids secreted by the adrenal cortex.

In the southern hemisphere, artificial lighting beginning in the first month of the summer was effective to maintain cyclicity of treated mares (Albrecht de David et al., 2011). Progesterone levels obtained during all months of the year in mares of this study confirmed absence of seasonal breeding. In seasonal breeding mares, progesterone is required to produce arachidonic acid, a precursor to PGF2α (Silvia et al., 1991). Low or absent P_4 concentrations resulted in compromised PGF2α response (Edgerton et al., 2000). The decrease in P_4 associated with the autumn transition might provide conditions for decreased PGF2 synthesis. However, the same decrease in P_4 concentrations was observed in normal-length estrous cycles where luteolysis occurred, and in spontaneously prolonged corpus luteum syndrome estrous cycles in which luteolysis failed. Moreover, this P_4

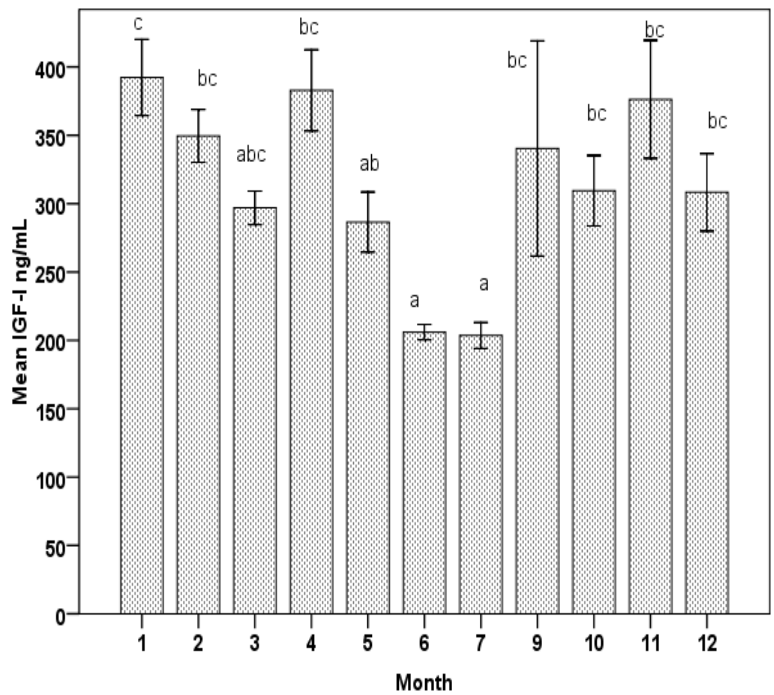

Figure 6. Mean levels Insulin like growth factor-I (ng/mL) during different months of the year. Different letters a, b and c indicates significance at P<0.05.

decline occurred in mares during a transition into the acyclic state as well as in mares that sustained estrous cycles throughout the winter. This suggests that declining P_4 concentrations do not directly provoke the spontaneously prolonged corpus luteum syndrome. However, in a recent study in which a dopamine antagonist was administered to mares during the time of the autumn transition, the seasonal decline in P_4 concentrations were prevented and the incidence of spontaneously prolonged corpus luteum syndrome did not increase during the autumn (King et al., 2008). Since no seasonality was not documented in mares of this study, so it seems that leptin plays no role and the decreased leptin levels during September and its increase during March, April and May in mares of this study may refer to the increased summer environmental temperature that decreases appetite of Arabian and imported horses which adversely affect body weight and body condition due to the mobilization of fat depot of the animals. In agreement to this current results, leptin levels increase according to body condition (Gentry et al., 2002). As well as, Cartmill et al. (2003) reported the presence of hyperleptinemic mares. As well as, concentrations of leptin generally vary directly with percent body fat (Chilliard et al., 2000; Prolo et al., 1998). In the horse, leptin concentrations were not only related to body condition but also to age, gender, and season (Cartmill, 2004; Fitzgerald and McManus, 2000; Gentry et al., 2002).

In seasonal breeding mares, increased concentrations of this hormone in circulation might be associated with the restart or maintenance of ovarian cyclicity in Lusitano mares (Ferreira-Dias et al., 2005). The hormone influencing cyclicity, leptin, is secreted from adipocytes and may serve as a signal from fat cells to the brain that reflects the adequacy of fat stores for reproduction (Cavinder et al., 2007). Mares having low levels of leptin became anestrus from November to April compared to those had continuous estrus. Mares had high leptin during follicular phase compared to luteal phase (Ferreira-Dias et al., 2005). Mares with high body condition continue to cycle during the winter and the presence of either large follicles or corpora lutae were not associated with high or low leptin concentrations (Fitzgerald et al., 2002; Gentry et al., 2002). Therefore, the follicular activity observed for mares with high body condition was likely due to the high body condition itself and not due to the elevated leptin concentrations

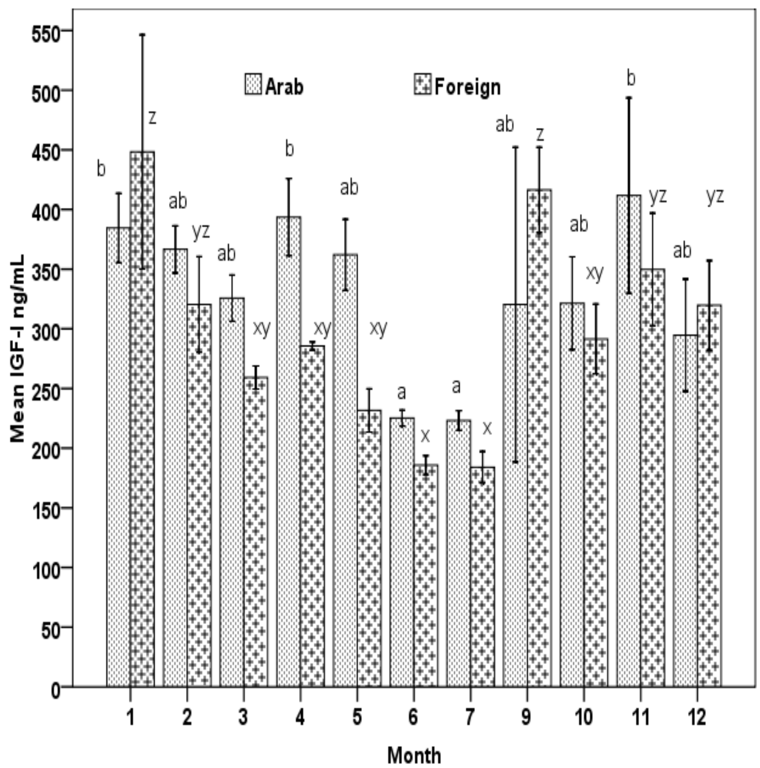

Figure 7. Mean levels Insulin like growth factor-I (ng/mL) during different months of the year. Different letters a, b and c of Arab mares and x, y and z of foreign mares indicates significance at P<0.05.

exhibited by some of those mares (Gentry et al., 2002). The presence of persistently high leptin concentrations was reported in about 35% of mares with high body condition, but not all mares of high body condition (Waller et al., 2006). Perturbations in leptin and insulin secretion observed in some high BCS mares are not associated with alterations in ovarian activity or the estrous cycle during winter and into the period of vernal transition (Waller et al., 2006).

Serum IGF- I

The decrease of IGF-1 levels during June and July in mares of this study, whatever their breed, may refer to the switching of the animals from good quality clover to another one with low quality with the exposing mares to heat stress decreasing their appetite so animals may transiently lose their body condition (Abo El-Maaty, 2011). Serum IGF-1 concentrations remained elevated for the mares in the fat-conditioned group (Cavinder et al., 2007). In agreement with the present results, circulating concentrations of IGF-I in transitional and cyclic mares, suggested that the somatotrophic axis is not involved in transition from anovulatory to ovulatory cycles (Watson et al., 2004). The role of growth factors may be significant with the ovary and the follicles, growth factors, in particular the insulin-like growth factor (IGF) system, are thought to play a key role in ovarian follicular growth and atresia (Armstrong et al., 2000). The IGFs have a variety of effects on follicular and luteal cells, including stimulation of steroidogenesis, via increased availability of steroid precursors and up regulation of steroidogenic enzyme expression and activity.

Follicular concentrations of IGF-I increase in large equine follicles (Bridges et al., 2002), whereas IGF-II concentrations are not different among different sizes of follicle. It has been suggested that intrafollicular IGF-I may be involved in selection of dominant follicles in mares (Donadeu and Ginther, 2002; Ginther et al., 2002). In mares fed with a low energy diet or protein during the winter, Sticker et al. (1995) observed diminished IGF-1 secretion, an important factor for follicular development. In mares, IGF-1 and its receptors increase specifically in the dominant follicle before divergence (Doyle et al., 2008), making it possible to attribute a special role to IGF-1 in the selection and subsequent development of ovulatory follicles (Ginther et al., 2004).

CONCLUSION

Breeding mares depends on the Latitudes they inhabit. Leptin and Insulin like growth factor-I have no role in breeding mares and their fluctuations depends on nutrient intake and body condition. Latitudes nearer to the equator had no great difference in photoperiod during different seasons of the year and so no effect of photoperiod on breeding mares was noticed. Breeding a mare in our Latitudes is a breeder decision.

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