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Extrato de pituitária equina obtido durante o verão melhora a dinâmica folicular de éguas durante o período de transição de outono

Equine pituitary extract obtained during summer improves follicular dynamics of mares during autumnal transition period

Extracto de pituitaria equina obtenido durante el verano mejora la dinámica folicular de las yeguas durante el período de transición otoñal

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Resumo

Para comparar a dinâmica folicular após o tratamento com baixas doses de extrato pituitário eqüino obtido durante o inverno e verão e estudar a relação entre dinâmica folicular e parâmetros ambientais, foram avaliadas 21 éguas acompanhando-se a dinâmica folicular e ovulação, do final da transição de primavera até o início da transição outonal. As éguas foram designadas para um dos tratamentos: Extrato Pituitário Eqüino verão (EPE-S), Extrato Pituitário Eqüino inverno (EPE-W), salina (Controle). Os tratamentos foram repetidos em Março/2017, após o equinócio outonal e Abril/2017. Temperatura, umidade, radiação solar e horas/luz/dia foram registradas e o Índice de Umidade e Temperatura (THI) foi calculado. Os folículos pré-ovulatórios atingiram o maior diâmetro (40,7 mm) em dezembro ($p<0,05$), após o aumento de luz/horas/dia. Os menores folículos pré-ovulatórios foram verificados em fevereiro (34,6mm) e março (35,0mm) ($p>0,05$). Após a EPE, os dias para ovulação diferiram, assim como o diâmetro dos folículos pré-ovulatórios ($p<0,05$). Éguas EPE-W apresentaram dinâmica folicular semelhante às Controle, ovulando folículos menores ($p<0,05$) e mais tarde ($p<0,05$) que éguas EPE-S. A temperatura e radiação solar não diferiram ($p>0,05$), mas a umidade e o THI sim ($p<0,05$). O THI não se correlacionou com o diâmetro dos folículos pré-ovulatórios ($p=0,37$), diâmetro dos folículos subordinados ($p=0,80$), ou número/folículos/onda ovulatória ($p=0,98$). Em conclusão, a duração do dia influenciou a função ovariana; baixas doses de EPE-S melhoram o crescimento folicular e taxa de ovulação na transição outonal; o diâmetro dos folículos pré-ovulatórios aumentou da primavera para o verão, mas o tempo necessário para a ovulação diminuiu.

Palavras chave: Estacionalidade reprodutiva equina; Gonadotrofinas; Período de transição anovulatório; Hormonioterapia.

Abstract

To compare follicular dynamics after treatment with low doses of equine pituitary extract obtained during winter and summer and to study the relationship between follicular dynamic and environmental parameters, 21 mares were evaluated to follow follicular dynamic and ovulation, from the final of the spring transitional up to the beginning of autumnal transitional period. Mares were randomly designed to one of three treatments: Equine Pituitary Extract from summer (EPE-S), Equine Pituitary Extract from winter (EPE-W) or saline (Control). Treatments were repeated twice: March/2017, after autumnal equinox and April/2017. Temperature, humidity, solar radiation and hours/ light/day were recorded and Temperature Humidity Index (THI) was calculated. Pre-ovulatory follicles reached the greatest diameter (40.7 mm) in December ($p<0.05$), following the increase in light/hours/day. The smallest pre-ovulatory follicles were verified in February (34.6 mm) and March (35.0 mm) ($p>0.05$). After EPE, days for ovulation differed, as well as the diameter of pre-ovulatory follicles ($p<0.05$). EPE-W showed similar follicular dynamics as Control, ovulating smaller follicles ($p<0.05$) and later ($p<0.05$) than EPE-S. Temperature and solar radiation did not differ ($p>0.05$), but humidity and THI differed ($p<0.05$). THI was not correlated to the diameter of pre-ovulatory follicles ($p=0.37$), diameter of subordinate follicles ($p=0.80$), or number/ follicles per ovulatory wave ($p=0.98$). In conclusion, daylight duration influenced ovarian function; low doses of EPE-S improvede follicular growth and ovulation rate during autumnal transition period of mares; pre-ovulatory follicle diameter increased from the spring to summer, but the time required to reach ovulation decreased.

Keywords: Equine breeding season; Gonadotropins; Transitional anovulatory period; Hormonal therapy.

Resumen

Para comparar la dinámica folicular usando dosis bajas de extracto de hipófisis equina obtenidas durante el invierno y verano y estudiar la relación entre la dinámica folicular y parámetros ambientales, se evaluaron 21 yeguas para seguir la dinámica folicular y ovulación, desde el final de la transición de primavera hasta el comienzo del período de transición otoñal. Las yeguas fueron diseñadas para los tratamientos: Extracto de pituitaria equina-verano (EPE-S), Extracto de pituitaria equina-invierno (EPE-W), salino (Control). Los tratamientos se repitieron em marzo/2017 (equinoccio de otoño) y abril/2017. Se registraron la temperatura, humedad, radiación solar y horas/luz/día y se calculó el Índice de Temperatura y Humedad (THI). Los folículos preovulatorios fueram mayores (40,7 mm) en diciembre ($p<0,05$), tras el aumento luz/horas/día. Folículos preovulatorios pequeños se verificaron en febrero (34,6 mm) y marzo (35,0 mm) ($p>0,05$). Después de la EPE, días para la ovulación y diámetro de los folículos preovulatorios difirieron ($p<0,05$). Yeguas EPE-W mostró dinámica folicular similar al Control, ovulando folículos pequeños ($p<0.05$) y más tarde ($p<0.05$) que EPE-S. La temperatura y radiación solar no difirieron ($p>0.05$); humedad y el THI sí ($p<0.05$). El THI no se correlacionó con el diámetro de los folículos preovulatorios ($p=0,37$), diámetro de folículos subordinados ($p=0,80$), número de folículos/onda/ovulatoria ($p=0,98$). En conclusión, la duración del día influyó la función ovárica; bajas dosis de EPE-S mejoran el crecimiento folicular y tasa de ovulación durante el período de transición otoñal; el diámetro de los folículos preovulatorios aumentó de la primavera al verano, pero el tiempo necesario para llegar a la ovulación disminuyó.

Palabras Clave: Estacionalidad reproductiva equina; Gonadotrofinas; Período anovulatorio de transición; Terapia hormonal.

1. Introduction

Mares are seasonally polyestrous, showing higher reproductive activity mostly between spring and summer and the environmental effect on reproduction is due the photoperiod (Williams et. al. 2012). This period is characterized by two equinoxes, which occur, in the South Hemisphere, on September, 21 (spring) and March, 21 (autumn), with the summer solstice (December, 21) in the meantime. Equinox determines equal light and dark, and in the spring, the days become longer than nights, while after the autumnal equinox, the nights become longer. On the other hand, after the summer solstice, or the longest day of the year, light hours decrease slowly. Since mares are long-day breeders, the ovarian activity, in the spring transition period, depends on the increased hypothalamic activity, and in the autumnal transition, on their capacity to maintain enough hypothalamic and pituitary activity, especially regarding LH secretion (Williams et. al. 2012, Morel, Newcombe & Hayward, 2010).

Seasonal anestrous duration varies among mares and, for the same female, also between years. This variation is due to inhibiting factors such as nutrition, body condition, weight gain or loss, social integration, ambient temperature, and reproductive history (Fitzgerald & MacMannus, 2000). During the transition from anestrous to the physiological polyestrous period, the mare will present estrous signs for many days without effectively developing ovulatory follicular waves or ovulation (Ginther et al. 2010). The autumn transition mirrors the spring transitional period. With time, besides the prolonged and irregular estrous, the follicles become larger and anovulatory. If the spring is the time to prepare the mares to become pregnant, the approach of autumn requires an acceleration of the entire process through hormonal manipulation to induce ovulation (Kwong & Klein, 2019).

To optimize the reproductive efficiency in mares some ovulation inducers, as the Human Chorionic Gonadotropin (HcG), Gonadotropin-Releasing Hormone (GnRH) and its analogs, and the Equine Pituitary Extract have been studied (Hyland et al., 1987; Ginther et al., 2009; Farias et al. 2010; Farias et al., 2019). Strategies as the administration of low-dose of GnRH, beginning near the autumnal equinox (Collins, Zieba & Williams, 2007), native GnRH associated with twice-daily injections of estradiol 17-β (O'Neil et al. 2019), GnRH agonist associated to naltrexone (Kwong & Klein, 2019) has been proposed to improve ovulation rate and pregnancy rates during the autumnal transition period.

Besides the photoperiod, environmental stress would impair reproduction in many species. As a long-day breeder the mare would be exposed to the higher temperature of the summer, increased solar radiation, and to the combination of ambient temperature and air humidity, which affects the thermal comfort (Hansen, 2009). Increased cortisol release induced by the exposition to stress does not affect reproductive functions of mares, because horses rapidly get accustomed to potential stressors (Aurich & Aurich, 2008).

This experiment was designed to test the hypothesis that the equine pituitary extract (EPE) obtained during the summer solstice would increase follicular diameter and improve ovulation rate at the beginning of the autumn transitional period. The main objectives were to compare follicular dynamics over the breeding season and after treatment with low doses of equine pituitary extract and to study the relationship between follicular dynamic and environmental parameters.

2. Methodology

The experiment was developed as a quantitative field research (Pereira et al. 2018). All management practices involving the mares were approved by the Animal Experimentation Ethics Committee, Universidade Federal de Goiás (process # 070-17).

2.1. Local, period and animals

This experiment was performed in the Brazilian tropical savannah, Goiás State, Latitude 16°40′48″ S, Longitude 49°15′18″ W, 750 meters above sea level, in the Southern Hemisphere, from September/2016 to April/2017. Twenty-one adult, non-pregnant and non-lactating light mares, aged between 5 and 8 years, healthy and with a body condition score of “2” to “4” on the five-point scale (Carroll & Huntington, 1988).

The mares were kept in pasture areas composed of *Pennisetum americanum* and Tifton 85, receiving mineral mixture and water *ad libitum*, supplemented with 1kg of commercial concentrate containing 15% protein added to 300 ml of vegetable oil, offered in individual feeders.

2.2. Reproductive evaluations

Evaluations started in September, during the first transition period, before the spring equinox. Mares were submitted to a daily protocol of gynecological examinations to verify the presence of a functional corpus luteum and/or follicles ≥20mm, until April, the second transition period, after the autumn equinox, by the confirmation of the first mare with longer estrous signals duration (above eight days) and slower follicular growth (same diameter in two consecutive evaluations). The mares were evaluated by rectal palpation and uterine and ovarian ultrasound examination (Mindray, DP–2200 Vet, 75L50EAV Endorectal transducer 5-10Hz). The number and size of follicles ≥20 mm was monitored, as well as the presence of a corpus luteum. The size of the structures was determined by the mean diameter of two linear measurements taken at a 90° angle to each other (Haadsma et al. 2007). The presence of follicles ≤ 10 mm was considered as follicular activity, while the verification of follicles ≥ 20 mm was considered as follicular divergence.

Interovulatory interval was recognized by the emergence of smaller follicular growth waves in the presence of a functional corpus luteum. The length of the estrous cycle was calculated by the time between two ovulations (Satué & Gardon, 2013). The uterine examination followed the observation of the edema degree, using scores (1= without edema, 2= median, and 3= maximum edema), adapted from Samper (2010), to access the effect of follicular growth on the endometrium and to assure that mares were cycling.

2.3. Equine pituitary extract and treatments

For the treatment with Equine Pituitary Extract (EPE), lyophilized and purified pituitary extracts obtained in the south region of Brazil during June/July (EPE-Winter) and December (EPE-Summer) prepared as described by Guilou & Combarnous (1983) were used (Laboratório de Reprodução Animal-Unicórnio-Brasília, Brazil).

Equine pituitary was collected at the latitude 30º20'11" S; longitude 54º19'12" W; altitude 114 meters. In the year of the collection, the temperature during summer varied from 15.8 to 30.1°C and, in winter, from 9.1 to 20.7°C. During the summer, the average light incidence was 13:33 hours and, during the winter, 10:50 hours (Airport Climatology Station, São Gabriel, INPE).

When the first mare began to show prolonged estrous signals (above eight days) and decreased follicular growth rate, it was considered the beginning of the autumnal transition period. Three days before the autumn equinox, all mares were treated with 0.375 mg of Cloprostenol Sodium (Ciosin®, MSD Saúde Animal), and were monitored for estrous signals.

Ovaries were daily evaluated until the verification of one or two follicles with diameter >30≤ 32 mm when the mares were randomly assigned to one of the treatments: EPE-S (n=7), Summer Equine Pituitary Extract; EPE-W (n=7), Winter Equine Pituitary Extract and Control (n=7), saline. Each mare received 125 IU of EPE or saline, i.m. every 12 hours until one or more follicles with 34/35 mm diameter were present when 500 IU of EPE were intravenously injected to induce ovulation. Ten days after ovulation Cloprostenol Sodium was repeated, and mares were submitted to the same EPE protocol.

2.4. Environmental parameters

Data regarding the ambient temperature (°C), relative humidity (%), solar radiation and Hours of Light per Day were daily obtained from the University Agrometeorological Station located 2.6 km from the reproduction center, and using the agroclimatological information system. Temperature and Humidity Index (THI) was calculated according to

Mader et al. (2006), following the equation: $THI = (0{,}8 \; x \; T^{\circ}) + [(UR/100) \; x \; (T^{\circ} - 14{,}4)] + 46{,}4$, where T° is the ambient temperature (°C) and RH s the relative humidity (%).

2.5. Statistical analysis

Data are presented as means ± SEM. Statistical analysis was performed by ANOVA followed by the “t” test. Pearson correlation was used to test the association between THI and the diameter of pre-ovulatory follicles, diameter of subordinate follicles, and the number of follicles per ovulatory wave. Data analysis was performed using the GraphPad Prism® 7 for Windows, and Microsoft Office Excel® 2010 was used to build the Figures.

3. Results and Discussion

3.1. Follicular dynamics during breeding season

Results regarding ovarian activity and number of hours of light per day from the end of the spring transitional period until the treatment with equine pituitary extract (EPE) at the beginning of the autumn transitional period are exposed in Table 1.

Table 1. Number of ovulations, days for ovulation, ovulatory follicle diameter (mm), follicle growth rate, subordinate follicle diameter (mm), number of follicles per ovulatory wave and hours of light per day (September/2016-April/2017, n=21 mares).

Month	Number of Ovulations *	Days for ovulation **	Ovulatory Follicle Diameter	Daily Growth Rate **	Subordinate Follicle Diameter	Follicles/ Ovulatory Wave	Hours/ Light/Day
Mean±SEM							
Sept	5±0.5	5.6±0.5	34.7±0.7^{a}	1.2±0.1^{a}	21.9±0.5	5.4±0.7^{a}	11.6±0.1^{a}
Oct	11±0.3	5.5±0.4	39.9±0.5^{b}	1.4±0.1^{a}	22.0±0.6	8.6±0.8^{b}	12.4±0.2^{ab}
Nov	25±0.3	5.7±0.2	39.8±0.7^{b}	1.4±0.1^{a}	22.4±0.4	9.1±0.8^{b}	13.2±0.3c
Dec	28±0.2	5.6±0.2	40.7±0.7^{b}	1.5±0.2^{b}	22.5±0.5	9.0±0.5^{b}	13.3±0.3^{ac}
Jan	18±0.5	5.8±0.3	36.6±0.5^{c}	0.8±0.1^{c}	22.0±0.5	7.5±0.7^{ac}	13.3±0.3^{ac}
Feb	15±0.3	5.8±0.2	34.6±0.6^{a}	0.5±0.1^{c}	22.4±0.6	7.5±0.7^{a}	13.2±0.4^{cd}
Mar	10±0.3	5.7±0.4	35.0±0.7^{ac}	0.5±0.2^{c}	21.7±0.5	4.5±0.9^{a}	12.5±0.3^{b}
Ap***							11.5±0.7^{a}
	P<0.0001	P=0.99	P <0.0001	P<0.0001	P=0.9287	P <0.0001	P<0.0001

^{a,b,c,d} within a columm indicates $p<0.05$. *differences for all the months; **from 32mm until ovulation; March, before treatment; ***only to compare Hours/Light/Day. Source: *The a*uthors.

In Table 1 it is possible to verify that, in December, the pre-ovulatory follicles reached the greatest diameter (40.7 mm), following the increase in light/ hours/day, as well as the number of ovulations (28), with 1.3 cycles/mare. Smallest diameters were verified in

February (34.6 mm) and March (35.0 mm), with no differences (p>0.05), although the reduction of hours of light per day was significant between these months (p<0.05).

Previously described data showed that for mares raised in the Northern Hemisphere, the mean ovulatory follicle diameter was 39.9 mm (range 22-50 mm), with significant negative effect (P<0.001) of the season (larger, 44.2 mm and smaller, 33.7 mm) from early spring to late summer, respectively (Morel, Newcombe & Hayward, 2010).

Duration and number of estrous cycles in mares vary according to the season, and it is inversely proportional to the length of the day, which means that cycles become shorter and are repeated at shorter intervals at the peak of the reproductive season (Satué & Gordon, 2013, Cuervo-Arango & Clark, 2010), which occurs during the late spring until the end of summer (between the spring equinox and summer solstice). Follicular activity in mares increases in the first half of the reproductive season due to the higher number of minor follicular waves and the higher concentration of gonadotropins (Donateu & Peterson, 2008).

In the summer, the duration of estrous decreases, probably by the acceleration of folliculogenesis before ovulation in response to the favorable photoperiod, reflecting the increment in the release of the gonadotropins, especially of LH (Ginther et al. 2018). The oocyte viability plays a critical role in reproductive success, and pieces of evidence suggest that it may be linked to the size of the pre-ovulatory follicle; the follicles must be bigger than 20mm in diameter for the included oocyte to achieve meiotic competence (Morel, Newcombe & Hayward, 2010).

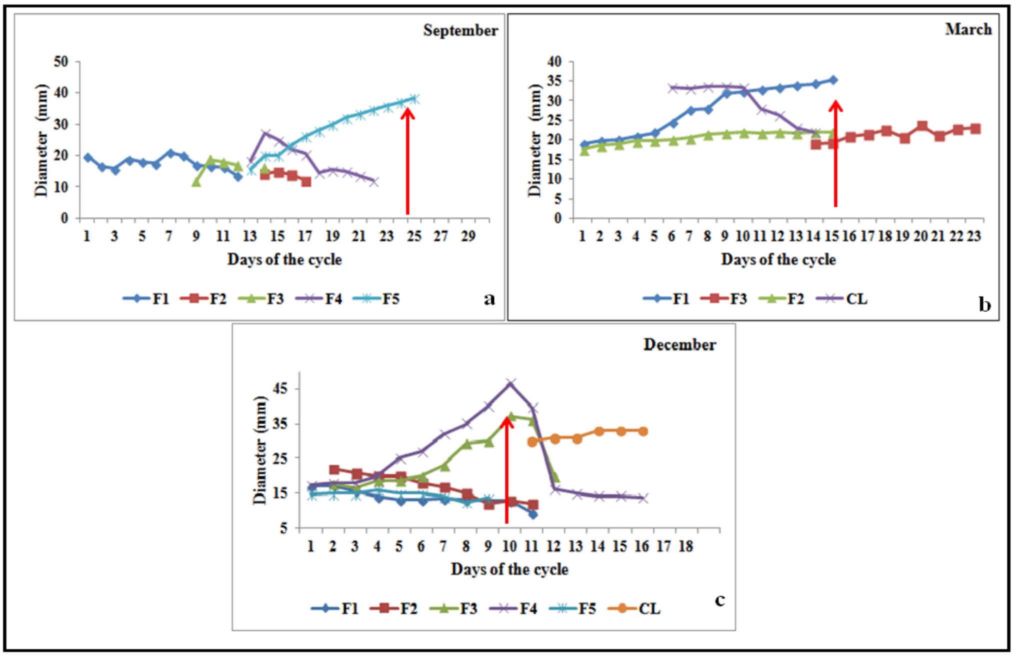
Another factor influencing the reduced follicle diameter is the occurrence of multiple ovulations (Satué & Gardón 2013), but in our study, double ovulations occurred only after treatment with EPE (Table 2). The emergence of a follicular wave occurs with about 10 small follicles (6 mm), followed by divergence when at least two follicles reach 20 mm; the ovulatory follicular wave ends when the so-called dominant follicle is about twice as large as the others (Ginther et al. 2018).

In Table 1 is shown that, although the pre-ovulatory follicle diameter increased during the season, reaching the maximum size in December when the summer solstice occurs and the longest hours/ light/day is reached, the diameter of subordinate follicles remained stable (from 21.7 to 22.5 mm), without differences (p>0.05), evidencing that, regardless of the period within the breeding season, once established the dominance the subordinate follicles no longer have the ability to continue their development (Ginther et al. 2018).

The number of follicles per ovulatory wave increased from the end of the spring transitional period (September), remained stable during the reproductive season and decreased with the beginning of the autumn transitional period, with differences ($p<0.001$), showing the effect of the environment through variation in the hours of light. From the divergence, the average daily follicular growth rate would be 3 mm (Ginther et al. 2017a, Ginther, 2017b), almost twice the higher growth rate described here (1.5 mm in December), indicating differences related to several factors such as breed and production system, that interfere in this parameter of follicular growth evaluation in mares.

Figure 1 summarizes follicular growth and ovulation in September and March.

Figure 1. Diameter of the largest follicle in the mares ovulating in September (a), March (b) and December (c), without day-to-day identity. ↑ indicates ovulation.



Source: The authors.

In Figure 1 it is possible to verify the effect of the month on the diameter of pre ovulatory follicle, during the spring and autumn equinox, respectively, when there is equal light and dark, and the mares are in spring (a) and autumn (b) transition, and in December, when, due to the summer solstice, there is the longest day of the year and, as a consequence, the maximum breeding activity.

3.2. Effect of EPE

From the autumn equinox (March) onwards, the mares were randomly divided into three groups and assigned to one of the treatments, which were repeated after cloprostenol-induced luteolysis in April. The results are exposed in Table 2.

Table 2. Days for ovulation, daily ovulation rate, diameter of the ovulatory follicle (mm) and number of ovulations after the two treatment cycles with EPE-W, EPE-S and Control.

	Days for Ovulation	Daily Growth Rate*	Ovulatory Follicle Diameter	Ovulation Number
First Treatment Cycle				
EPE-W (n=7)	6.4±0.8^{a}	1.9±0.1^{a}	35.3±0.6^{a}	4
EPE-S (n=7)	4.7±0.7^{b}	1.9±0.1^{a}	40.9±0.9^{b}	10**
Control (n=7)	8.0 ±0.8^{c}	0.5±0.08^{b}	34.9±0.7^{a}	4
	P = 0.005	P< 0.001	P = 0.002	
Second Treatment Cycle				
EPE-W (n=7)	5.6±0.8^{a}	0,4±0.09^{a}	34.3±0.5^{a}	2
EPE-S (n=7)	4.2±0.7^{b}	1,9±0.1^{b}	39.8±0.6^{b}	8**
Control (n=7)	-		-	-
	P = 0.001	P= 0.001	P = 0.02	

^{a,b} within a columm indicates p<0.05. EPE-W= Equine Pituitary Extract- Winter, EPE-S= Equine Pituitary Extract- Summer. *From 32mm until ovulation. ** Double ovulations. Source: The authors.

In Table 2 it is possible to verify that the number of days for ovulation differed between treatments (p<0.05), as well as the diameter of the pre-ovulatory follicle (p<0.05). EPE-W mares showed a similar follicular dynamics as control mares; ovulated smaller follicles (p<0.05) and later (p<0.05) than EPE-S mares. Daily follicle growth rate was the same for EPE-W and EPE-S mares in the first treatment cycle, with a difference for control mares (p<0.05), but the number of days to reach the pre-ovulatory diameter was significantly lower for EPE-S mares, showing the effect of the pituitary extract obtained in summer on the ovulatory follicle quality. For the second treatment cycle the daily follicle growth rate differed between EPE-W and EPE-S (p<0.005), and no control mare ovulated. EPE contains FSH and LH, but due to the extraction method the standardization of the proportion of each of these hormones is difficult to measure, being accepted that extracts of equine pituitary contain approximately 60% of LH and 40% of FSH, but the season in the region where EPE is obtained influences the amount and proportion of these hormones. According to the manufacturer, in previous trials related to the accuracy of dose-effect relations, it was established that 1 mg of EPE corresponds to 500 IU of gonadotropins, and each pituitary generates 1.2 mg of extract of each gonadotropin (FSH and LH); each 1 mg contains between 3 and 10 IU (Laboratório Unicornio LTDA).

Concentration of FSH extracted from mare pituitary during or not the reproductive season did not differ, but that of LH was markedly influenced by the season (increasing from spring to summer and decreasing from autumn to winter) (Hart et al. 1984), probably explaining our findings, since for the present study EPE was obtained from mares raised in a region with marked seasons through the year.

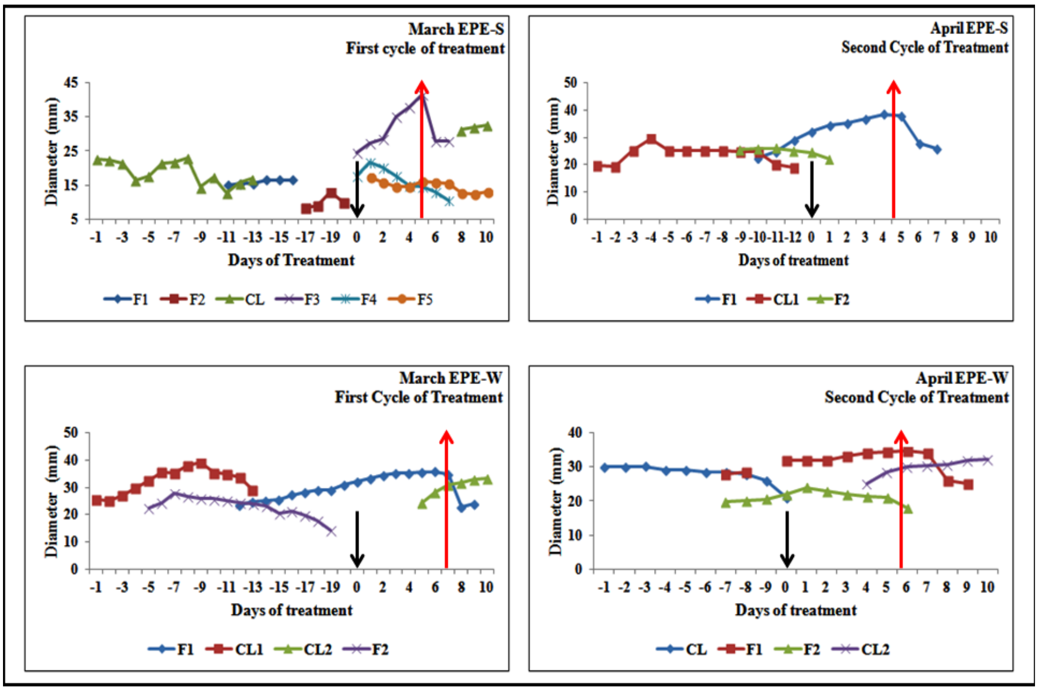
Melatonin, released by the pineal in response to the perception of lower intensity and light duration in the environment, is the modulator of the frequency and amplitude of gonadotropin-releasing hormone (GnRH), which affects FSH and LH pituitary secretion in horses. Gonadotrophic cells are located in the distal and tuberal pars of the pituitary and the heterogenicity in the storage pattern of LH and FSH in the gonadotrophic cells population is the morphological basis for the differential regulation of gonadotropin secretion during the reproductive cycle. In cyclic mares, the density of gonadotrophic cells in tuberal pars is four to five times higher than in anovulatory mares (Aurich 2011). Considering that the evaluated follicles already passed the divergence phase, they were, therefore, LH-dependent follicles; the reduction in the FSH concentration is necessary for the divergence, an event in which there is no LH involvement. However, this gonadotropin is essential for the growth and maturation of the future ovulatory follicle.

In mares, there is no abrupt pre-ovulation LH episode, and the concentration of LH remains high even after ovulation (Ginther et al. 2010). Thus, in EPE-S mares, the follicles that went through the divergence were probably exposed to the higher amount of LH present in the extract obtained in the period of maximum luminosity, and this exposure accelerated the growth and increased the diameter of the pre-ovulatory follicle. Another interesting point is that, although the treatment was not proposed to promote more than one ovulation, three EPE-S mares in the first cycle and one in the second showed double ovulation.

Equine pituitary extract has been used to induce multiple ovulations in embryo donor mares, and there is a consensus among researchers that it is a good option for this purpose, as well as to induce ovulation (Faria & Gradela, 2010), also in low doses, inducing more than one ovulation per cycle (Bonin et al. 2010).

The fact that all EPE-S mares have ovulated in both cycles, as well as the occurrence of double ovulations in both cycles, suggests that EPE obtained during summer are more effective when used in low doses. Figure 2 summarizes the results regarding follicular grow and ovulation during the two treatments cycles.

Figure 2. Diameter and ovulation time for EPE-S and EPE-W in the two treatments cycles. ↓indicates the beginning of treatment; ↑indicates ovulation.



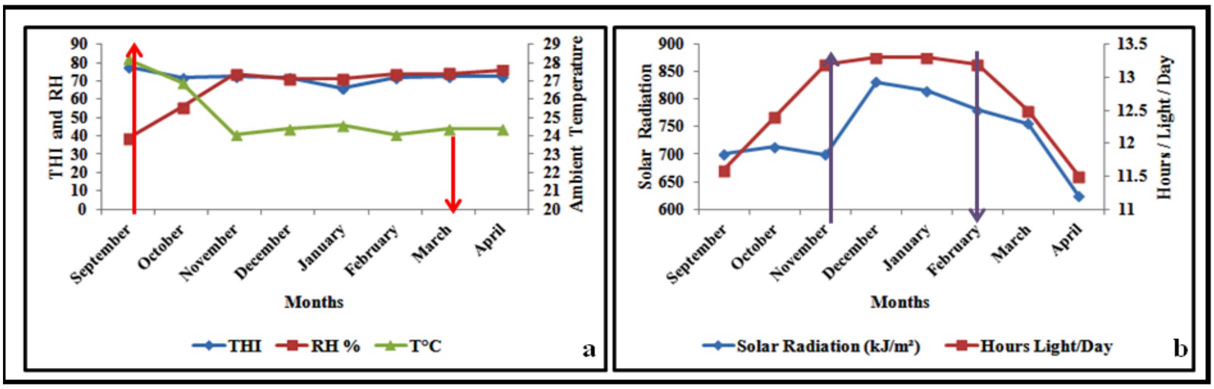
Source: The authors.

In Figure 2 the time between treatment and ovulation is pointed by the arrows and evidences the improvement of growth velocity after the use of EPE-S in both months, March and April.

3.3. Effect of environmental parameters

The environmental parameters were monitored during the experimental period to evaluate whether, as with other species, it could influence ovarian activity in heat stress situations (El-Maaty, 2011, Hansen, 2009). In the Figure 3 are exposed the Temperature and Humidity Index (THI), ambient temperature (T°C), air relative humidity (RH%), solar radiation, and hours/light/day.

Figure 3. Temperature and humidity índex (THI) ($p<0.05$), relative humidity (%) ($p<0.05$) and ambient temperature (°C) ($p>0.05$), (a). Solar radiation (kJ/m^2) ($p>0.05$) and hours/light/day ($p<0.05$), (b). ↑↓indicates highest and lowest ambient temperature. ↑↓ indicates the period of increased hours/light/day.



Source: The authors.

In Figure 3 the variations of environmental parameters over the months are shown. Means were calculated based on the days between the follicular divergence and ovulation and also for the months, as well as the daily and monthly means of solar radiation, since the mares remained in pasture regime all the time. The ambient temperature and solar radiation did not differ between months ($p>0.05$), but the relative humidity and THI differed ($p<0.05$). There was no significant correlation between THI in the period from divergence to ovulation and the diameter of pre-ovulatory follicles ($p=0.37$), the diameter of subordinate follicles ($p=0.80$), and the number of follicles per ovulatory wave ($p=0.98$), suggesting that this index did not interfere with these variables. Heat, exercise under hot and humid conditions, transportation, handling and change in environment would increase the circulating levels of stress hormones and interfere with the reproductive hormones in horses, impairing ovarian function and causing adverse effects on reproductive performance of mares (Pessoa et al. 2011, Vasquez et al. 2010). The findings that, in our study, THI did not interfere with the follicular dynamics may be related to the fact that the mares included in the experimental groups were breeding mares and not subjected to any extra physical activity during the experiment, and, as previously reported, were well adapted or accustomed to the environmental potential stressors (Aurich & Aurich, 2008). Our data suggest an individual, more than the environmental effect on the follicular dynamics of mares during the breeding season.

4. Final Considerations

In this experimental research we aimed to answer an important question about reproductive cyclicity of mares and how the autumnal transition period could be better used by equine specialized veterinarians. The finding that the time of pituitary glands collection impacts the ovarian response will open a new window about the use of equine pituitary extract in the mare breeding management practices. It was possible to prove the initial hypothesis that it would be possible to increase follicular diameter and improve ovulation rate of mares during the autumn transitional period.

In the current study, the objectives were achieved since obtained data allowed concluding that the use of low doses of equine pituitary extract obtained during the month of summer solstice in the Southern Hemisphere was effective in improving follicular growth and ovulation rate at the beginning of the autumnal transition period of mares. Pre-ovulatory follicle diameter increased from the spring to summer, but the time required to reach ovulation decreased. Among the environmental evaluated parameters, only daylight duration influenced ovarian function. Further studies would be performed to evaluate how long over the transitional period the mares will be responsive to the equine pituitary extract.

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