



EFFECT OF SEASONAL ENVIRONMENTAL CHANGES ON SELECTED REPRODUCTIVE PARAMETERS IN MARES

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Summary

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The aim of this study was to monitor and evaluate the seasonal changes on selected reproductive parameters in mares. Ninety-six mares of different breeds, aged 3 to 23 years were evaluated during the breeding season 2015–2018 at the Equine clinic at University of Veterinary Medicine and Pharmacology in Košice, Slovakia (48°42'N, 21°15'E). The beginning of the estrus was determined by history or observation, mares were examined every 6 hours, blood for progesterone analysis was taken from the jugular vein. Correlation analyses were performed using both the Pearson and the Spearman correlation coefficient. Statistical analysis was performed using the functions of Microsoft Excel and GraphPad Prism. The day length ($r = -0.708$, $P < 0.0001$) and the average ambient temperature ($r = -0.754$, $P < 0.0001$) had a statistically significant effect on the duration of estrus. The shortest estrus was recorded in July with day length of 15 hours 40 minutes at an average temperature of 21.4 ± 0.52 °C, with duration 4.67 ± 0.58 days. The longest estrus was recorded in April with day length of 11 hours 48 minutes at average temperature of 6.9 °C. The environmental factors did not affect the size of the preovulatory follicles, the concentration of progesterone, the internal changes of uterus and the external manifestations of estrus.

Key words: estrus, mare, ovulation

INTRODUCTION

Mean length of the estrous cycle in the mare population during the physiologic breeding season is approximately 21 days, but it can vary greatly (range 18–24 days) (Raz & Aharonson-Raz, 2012). However, although estrus typically comprises 4–7 days of the cycle, its length is more vari-

able (ranging from 4–14 days or more) (Santos *et al.*, 2015). In the beginning and at the end of the breeding season the length of estrus may be 7 to 12 days, whereas, around the summer solstice, estrus may last only 3 to 4 days. Therefore, the duration of estrus is shortest during the peak of the ovulatory season (Raz &

Aharonson-Raz, 2012). In pony breeds, the duration of estrous cycle is in average two days longer (Aurich, 2011). In practice, the size of the ovulating follicle is often considered as an individual parameter of each mare (Lefrançois & Bruyas, 2016). The seasonal reproductive pattern in mares is the result of a circannual endogenous rhythm (Fazio *et al.*, 2009). Resumption of ovarian activity during the spring transition period is associated with gradually increasing hypothalamic activity as the suppressive effects of melatonin are lifted (Nagy *et al.*, 2008). As the mare progresses through the transition period pre-ovulatory follicle oestradiol production increases (Watson *et al.*, 2003), this may be a reflection of increasing pre-ovulatory follicle size and function (Morel *et al.*, 2010).

The general seasonal pattern is that the incidence of ovulation is minimal or absent during the winter, transitionally increasing during the spring, maximal during the summer, and transitionally decreasing during the fall. The low incidence of ovulation for the winter months reflects anovulatory seasons of different lengths among individual mares combined with uninterrupted ovulation throughout the year by some mares. Except for a few studies in the past few years, data on the seasonal distribution of ovulations have been obtained from the northern and southern temperate zones, as in the examples. These zones begin at approximate latitudes of 23°N and 23°S, respectively, where the difference in length between the longest and shortest day is about two hours (Ginther *et al.*, 2004). In a slaughterhouse study done in southern Mexico (15°N–22°N), a definitive seasonal ovulatory pattern was found; about 10% of the reproductive tracts had indications of ovulation in January and February and close

to 100% in July and August (Saltiel *et al.*, 1982). The slight seasonal daylength variation at 10°N in Venezuela (longest daylength, 12 h and 46 min) was also associated with a seasonal reproductive pattern. Changing daylength is the primary controller of the follicle dynamics of seasonal reproductive rhythms (Quintero *et al.*, 2000; Ginther *et al.*, 2004).

The aim of this study was to monitor and evaluate the effect of day length and ambient temperature on the duration of estrus, size of preovulatory follicle, progesterone concentration, uterine pattern and external signs of estrus in mares.

MATERIALS AND METHODS

Animals and location

During the breeding season (February – September) in years 2015, 2016, 2017, 2018, 99 mares aged 3–23 years were evaluated, and three of them were excluded from the study due to the pathological condition of reproductive system. The mares were hospitalised and all of them were inseminated at the Equine Clinic of the University of Veterinary Medicine and Pharmacy in Košice (48°42'N, 21°15'E). Breed representation consisted of Slovak warmblood horse, other warm-blooded breeds bred in Germany, Netherland, France, Hungary, American Quarter Horse and Hucul. Mares were housed in individual indoor boxes with smaller outer paddles, fed hay and grain. The mares included in the study had not been administered any drugs known to accelerate ovulation.

Estrus detection

Beginning of the estrus was determined by history and observation of external signs. Mares were examined every six hours

during late estrus by rectal palpation and ultrasonography to determine the time of ovulation. The endpoint of estrus (ovulation) was determined by the time of artificial insemination of the mare, with an accuracy of 0–6 hours.

A subjective modified scoring system (1 – 3) previously described by Pycock (2002) for the degree of intensity of external changes and behaviour of mares was established. A grade of 1 was given to mares with mild or absent symptoms, rare urination, passive behaviour. A grade of 2 was given to mares with distinct symptoms, more frequent urination with winking, hyperaemic vulva, slight interest to teaser. A grade of 3 was given to mares with strong interest in teaser, frequent urination, winking and squatting, tail deviation. The results present data from last examination.

Ultrasonic examination

Uterus and ovaries of the mares were examined by palpation and ultrasound scanning per rectum, using a ultrasonograph Aloka SDD - 50 0 (Tokyo MURE HITAKA – SH Co., Ltd. Japan) with a linear rectal probe UST-588-U, 0.5 MHz. Detection of the preovulatory follicles size, the presence of endometrial folds and mucus in uterus was performed ultrasonographically.

The size of the preovulatory follicle in centimeters was determined by measuring its largest diameter. The measurement was repeated three times and the mean follicle size was calculated from the measured values.

A subjective modified scoring system (1 – 3) described previously by Samper (1997) for the degree of endometrial oedema was established. A grade of 1 was given to mares that had significantly decreased uterine oedema, without endo-

metrial folds. A grade of 2 was given to mares with visible endometrial oedema and presence of mucus in the uterus. A grade of 3 was assigned to mares with maximal uterine oedema, good visible endometrial folds and good visible presence of mucus in the uterus. In order to use this scoring system the mares were examined on a regular basis throughout their estrous cycle and the pattern of endometrial edema determined. The results present data from last examination.

Progesterone analysis

To determine the progestogen concentration, a blood sample was taken from the *vena jugularis* into sterile BD Vacutainer plastic tubes with a clot activator. Serum was separated and stored at –20 °C until assayed for progesterone. Only blood samples collected during last evaluation (in time of insemination) were evaluated. Samples were analysed by Architect 2000i from ABBOTT, CLIA method – chemiluminescent immunoassay in a specialised RIA laboratory in Košice. Progesterone concentration values were determined with a sensitivity of 0.10 to 400 ng.mL⁻¹. Data were expressed as a mean ± standard deviation.

Day length and ambient temperature

Measurements of the Slovak Hydrometeorological Institute in Košice were used to determine the average temperature in a given month and the length of sunshine on the 15th day of the month.

Statistical analysis

Statistical analysis was performed using the functions of Microsoft Excel and GraphPad Prism. Correlation analyses were done using the Pearson correlation coefficient for correlation between environmental factors and duration of estrus,

preovulatory follicle size, progesterone levels. Spearman correlation coefficient was used for correlation between environmental factors and uterine pattern and external signs of estrus. P values <0.05 were considered significant. For comparison of proportions between uterine pattern and external signs of estrus "N-1" Chi-squared test was used and P values < 0.05 were considered to be significant.

RESULTS

Effect of day length and ambient temperature on duration of estrus

During the years 2015 to 2018 the mean duration of estrus was 5.70±1.22 days. The shortest estrus was recorded in July – its average duration was 4.67±0.58 days.

The average ambient temperature in this month was 21.6±0.52 °C and the daylength on the 15th day of July was 15 hours 40 minutes. The longest average duration of estrus was recorded in March – 8.33±0.91 days. The average ambient temperature in March was 6.9±0.67 °C and the daylength was 11 hours 48 minutes during these years. There was a negative correlation between environmental factors and estrus duration. With increasing ambient temperature, the duration of estrus was shorter (r = -0.754, P<0.0001); similarly, with increasing daylength duration, the duration of estrus decreased (r = -0.708, P<0.0001) (Table 1).

Effect of day length and ambient temperature on preovulatory follicle size

The average preovulatory follicle size during the entire season of 2015–2018 was

Table 1. Relationship between environmental factors and reproductive parameters. Data are presented as mean ± SD.

Month of breeding season	Day length (min)	Average temperature (°C)	Estrus duration (days)	Follicular size (cm)	Concentration of progesterone (ng/mL)
March	708.33 ± 0.47	6.9 ± 0.67	8.33 ± 0.91	5.40 ± 0.08	0.32 ± 0.03
April	817.08 ± 1.04	12.3 ± 2.27	7.25 ± 2.27	4.92 ± 0.30	0.36 ± 0.13
May	910.54 ± 0.63	17.1 ± 1.54	5.88 ± 0.93	5.09 ± 0.49	0.32 ± 0.13
June	963.00 ± 0.00	20.2 ± 0.40	5.11 ± 0.69	4.97 ± 0.41	0.32 ± 0.13
July	940.22 ± 0.42	21.6 ± 0.52	4.67 ± 0.58	4.95 ± 0.40	0.33 ± 0.12
August	857.43 ± 1.29	21.5 ± 1.71	5.43 ± 0.50	5.13 ± 0.25	0.42 ± 0.14
September	752.67 ± 0.47	16.4 ± 0.75	7.33 ± 0.94	5.00 ± 0.14	0.33 ± 0.12
Mean values during the entire study			5.70 ± 1.22	5.02 ± 0.41	0.33 ± 0.13
Correlation and significance level			r ^{ac} = -0.708 P ^{ac} < 0.0001	r ^{ad} = -0.096 P ^{ad} > 0.1	r ^{ae} = -0.090 P ^{ae} > 0.1
			r ^{bc} = -0.754 P ^{bc} < 0.0001	r ^{bd} = -0.059 P ^{bd} > 0.1	r ^{be} = 0.055 P ^{be} > 0.1

ac – relationship between daylength and estrus duration; ad – relationship between daylength and follicular size; ae – relationship between daylength and progesterone concentration; bc – relationship between ambient temperature and estrus duration; bd – relationship between ambient temperature and follicular size; be – relationship between ambient temperature and progesterone concentration.

Effect of seasonal environmental changes on selected reproductive parameters in mares

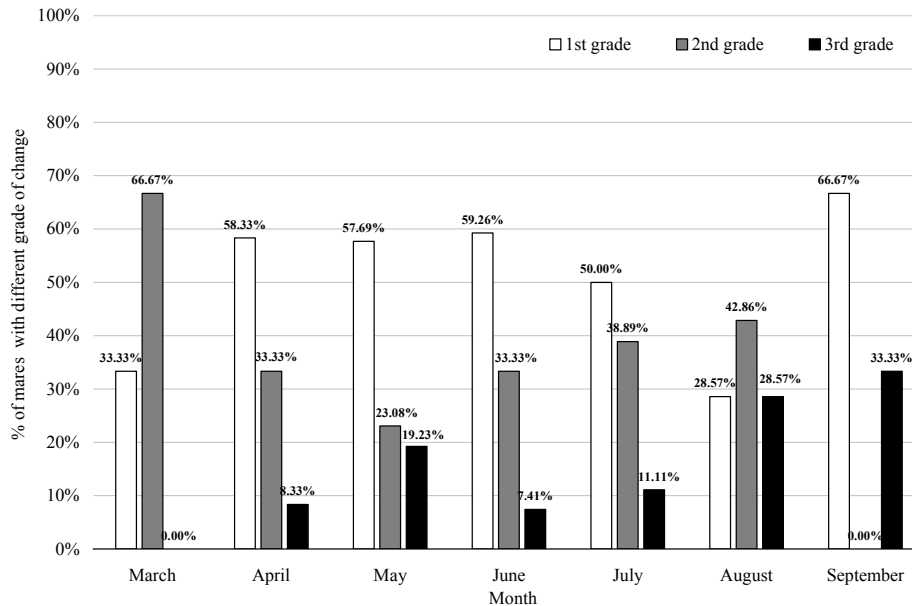


Fig. 1. Changes in uterine pattern during entire period of study.

5.02 ± 0.41 cm. The smallest average size of preovulatory follicles was recorded in April – 4.92±0.30 cm. The highest average size of preovulatory follicle was observed in March – 5.4±0.08 cm. The correlation between mean ambient temperature and preovulatory follicle size ($r = -0.059$, $P=0.566$), day length and preovulatory follicle size ($r = -0.096$, $P=0.350$) was not statistically significant using the Pearson correlation coefficient (Table 1).

Effect of day length and ambient temperature on concentrations of progesterone

The average progesterone concentration over the entire observation period was 0.33±0.13 ng.mL⁻¹. The lowest mean progesterone was recorded in March – 0.32±0.03 ng.mL⁻¹, May and June – 0.32±0.13 ng.mL⁻¹. The highest average progesterone concentration was recorded in August – 0.42±0.14 ng.mL⁻¹. The correlation

between mean ambient temperature and progesterone concentration ($r=0.055$; $P=0.594$), daylength and progesterone concentration ($r = -0.090$, $P=0.385$) was not statistically significant using the Pearson correlation coefficient (Table 1).

Effect of day length and ambient temperature on the uterine pattern

Throughout the study period, first-grade changes in the uterus was observed in 54% (52 mares), 2nd grade in 32% (31 mares) and 3rd grade in 14% (13 mares). The correlation between mean ambient temperature and changes of uterine pattern ($r=0.030$, $P=0.770$), daylength and changes of uterine pattern ($r=-0.069$, $P=0.503$) was not statistically significant using the Spearman correlation coefficient. Fig. 1 shows the number of mares and the degree of change during each month of the season during the entire study period.

Effect of day length and ambient temperature on the external signs of estrus

During entire period first-grade external symptom intensity was observed in 9% of mares (n=9), 2nd grade in 35% (n=34) and 3rd grade of external symptoms – in 55% (n=53) of mares. The correlation between average temperature and external signs of estrus ($r = -0.074$, $P=0.476$), daylength and external signs of estrus ($r = 0.048$, $P=0.645$) was not statistically significant using the Spearman correlation coefficient. Fig. 2 shows the number of mares and the intensity of the external signs during each month of the season during the entire period.

Relationship between uterine pattern and external signs of estrus

By using "N-1" Chi-squared test was concluded that in some months the uterine pattern had an influence on external signs of estrus. In months April – July, when 1st grade intensity of changes in

uterine pattern was of higher incidence, we noticed lower incidence of 1st grade of intensity of external signs of estrus. In these months we noticed that lower incidence of 3rd grade of intensity of changes in uterine pattern was related with higher incidence of 3rd grade intensity of external signs of estrus. Results are presented in Table 2.

DISCUSSION

In mares, it is well documented that reproductive processes are affected by the seasonal changes in photoperiod. The peak of the ovarian function occurs in the summer months (June, July, August in northern hemisphere), is absent or minimal during winter (December, January, February), and is characterised by irregularity during spring (March, April, May) and fall transitional periods (September, October and November in northern hemisphere) (Ishak, 2017). Only very little

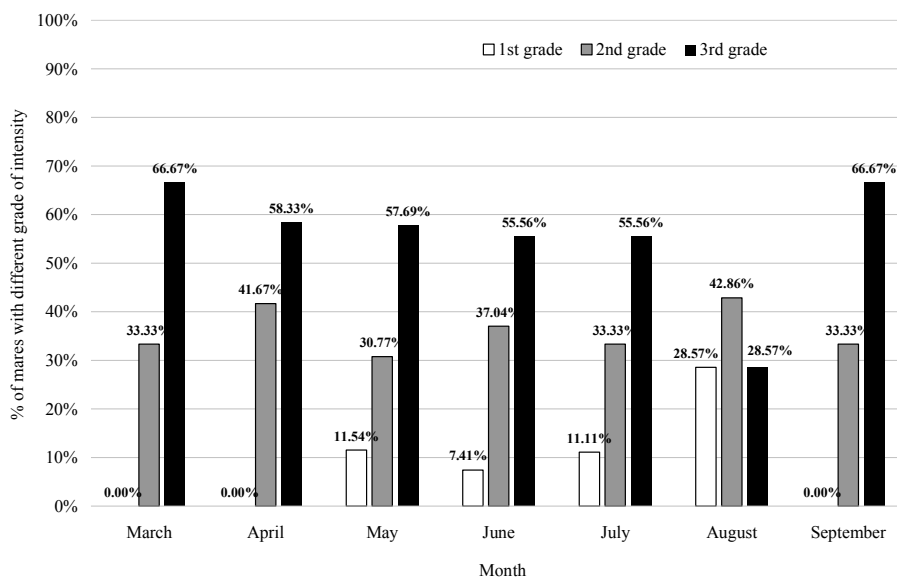


Fig. 2. Changes in external signs of estrus during entire study.

Table 2. Relationship between environmental factors, uterine pattern changes and changes of external signs of estrus

Month	Sample size	1st grade			2nd grade			3rd grade		
		Changes in uterine pattern	Intensity of external signs	P	Changes in uterine pattern	Intensity of external signs	P	Changes in uterine pattern	Intensity of external signs	P
March	3	33.33%	0.00%	NS	66.67%	33.33%	NS	0.00%	66.67%	NS
April	12	58.33%	0.00%	P<0.05	33.33%	41.67%	NS	8.33%	58.33%	P<0.05
May	26	57.69%	11.54%	P<0.05	23.08%	30.77%	NS	19.23%	57.69%	P<0.05
June	27	59.26%	7.41%	P<0.05	33.33%	37.04%	NS	7.41%	55.56%	P<0.05
July	18	50.00%	11.11%	P<0.05	38.89%	33.33%	NS	11.11%	55.56%	P<0.05
August	7	28.57%	28.57%	NS	42.86%	42.86%	NS	28.57%	28.57%	NS
September	3	66.67%	0.00%	NS	0.00%	33.33%	NS	33.33%	66.67%	NS

P – level of significance; NS – not significant (P>0.05).

current studies are available with accurate and exact data on the average temperature of the environment and the day length, especially in similar climatic conditions. In our study, the mean duration of estrus during entire breeding season was 5.70±1.22 days. In a study published by Popova (2015), the average duration of estrus during the summer months in Bulgaria (43°16'N) was 3.83±0.74 days. In a study presented by Lodi *et al.* (1995) mean lengths of estrus were 7.5±2.3, 6.5±1.8, and 7.1±1.4 days. They performed their observation during December, January and February and the study site was situated at 19°13" south latitude and 45° west longitude in Brazil. Ambient temperature ranged from 13 °C to 33 °C. In study presented by Dowsett *et al.* (1993), the mean duration of estrus was 7.5 days (SEM=0.4). The lowest incidence of estrus occurred at the time of the winter solstice (June 22) in the Southern Hemisphere. Winter estruses (mean=9.3 days, SEM=0.8) were longer than those of summer (mean=6.6 days, SEM=0.5) and autumn (mean=6.6 days, SEM=0.9). The study was conducted in South-East Queensland (27°27' South latitude)

(Dowsett *et al.*, 1993). This study was also performed during the entire year not only in breeding season.

Estrous cycle is an interaction of hormones of the pineal gland, hypothalamus, pituitary, gonad and endometrium lasting 21 days (Cortés-Vidauri *et al.*, 2018). Seasonal effect on mare's estrous cycle was confirmed by few authors. Study published by Mateu-Sánchez *et al.* (2016) has shown that the length of the follicular phase was influenced (P<0.001) by the month of season. The mean length of the follicular phase with estrus-like echotexture was highest at the beginning of the season and then decreased gradually to the end of the season (Mateu-Sánchez *et al.*, 2016). In our study we confirmed the influence of season on estrous cycle.

Photoperiodic regulation and changes in the hypothalamic-hypophyseal axis of mares are usually associated with the seasonal reproductive activity of mares in the temperate but not in subtropical areas (Ali *et al.*, 2014). These differences could be due to different environmental conditions in which these studies were performed. In a study published by Quintero *et al.* (1995) during one year; the average length

of estrus during entire study was 5.8 ± 2 days. The lengths of both the estrous cycle and estrus were significantly ($P < 0.001$) affected by season. Estrous cycle and estrus were longer in January for estrous cycle (22.2 ± 5.8 days) and in December for estrus (7.7 ± 1.4 days) with day length 11 hours and 33 minutes. Estrous cycle and estrus were shorter in July for estrous cycle (13.4 ± 1.8 days) and in April for estrus (3.6 ± 1.9 days) with day length 12 hours and 20 minutes. Except for a slight rise ($P < 0.005$) in May, estrous cycle and estrus lengths remained constant from April through September. These results shows similarity with ours. In our study, the shortest duration of estrus was observed in July. Statistical analysis shows significant influence of photoperiodism to duration of estrus ($r = -0.83$; $P < 0.0001$) (Quintero *et al.*, 1995). In our study we confirmed influence of photoperiodism to estrus duration ($r = -0.708$; $P < 0.0001$).

The role of environmental temperature as a signal for reproductive activity in poikilotherms (particularly reptiles and amphibians) is well recognised, but data relating to mammals is sparse and do not generally distinguish between temperature and photoperiodic effects (Guerin & Wang, 1994).

Some work has claimed follicular diameter to be a good predictor of time of ovulation when using a certain type of breed. However, the great range of follicular preovulatory diameter in the mare, 24 h prior to ovulation from 34 to 70 mm renders this criterion unreliable to estimate the optimal breeding time. This large range is mainly due to factors such as time of year with larger follicles early in the season (April–June), number of preovulatory follicles (double preovulatory follicles are smaller than single), breed (large draught breeds such as Clydesdale, Shire,

Irish Draught, Welsh Cob with larger follicles than ponies, Thoroughbred (TB) or Standardbred) and individual variation within a breed (Cuervo-Arango & Newcombe, 2008). The follicles are larger in April (46 mm) and May (48 mm) compared to July (40 mm) (Ginther & Pierson, 1989). In this study, we measured preovulatory follicle size and evaluated the influence of photoperiodism and ambient temperature to follicular size. In our results there is no sufficient evidence to conclude there is a relationship between day length ($r = -0.096$, $P > 0.05$) and ambient temperature ($r = -0.059$, $P > 0.05$), and preovulatory follicular size. In a study by Morel *et al.* (2010), preovulatory follicle size was observed in 1,492 Thoroughbred mares. Mean preovulatory follicle size was 3.99 ± 0.48 cm, the largest follicle was observed in February (4.42 ± 0.35 cm) and the smallest mean follicular size was observed in August (3.37 ± 0.48 cm). Authors affirmed that preovulatory follicular size was influenced by season ($P < 0.001$). In our study, the largest mean follicular size was observed in March, the smallest mean follicle was observed in April. These differences may be caused by small group of mares with ovulation during these months (3 in March, 12 in April).

Progesterone concentration is different during the estrous cycle, lower concentration is measured at beginning of follicular phase (0.48 ± 0.2 ng.mL⁻¹) and stays below 1 ng.mL⁻¹ during 4.7 ± 0.2 days before ovulation (Reed *et al.*, 2017). In our study the average progesterone was 0.33 ± 0.13 ng.mL⁻¹ throughout the study.

During the estrous cycle, the uterus, vagina and endometrium of the mare undergo pronounced changes related to variations in the endocrine milieu. They can easily be differentiated by clinical examination. During estrus, high concen-

trations of estrogen and low concentrations of progesterone contribute to increasing uterine wall oedema together with opening of the cervix and flattening of uterus and vagina. Histologically, the endometrial oedema is most apparent in the stratum compactum. It is often associated with accumulation of varying small amounts of fluid within the uterine lumen (Aurich, 2011). According to McKinnon *et al.* (1987) a reduction in endometrial folding from three to zero indicates approaching ovulation when a three point scale is used. In our results there is no sufficient evidence to conclude that there is a relationship between day length ($r = -0.069$, $P > 0.05$) and ambient temperature ($r = 0.030$, $P > 0.05$) and the uterine changes in time of ovulation.

Estrous period of the mare usually lasts 4–7 days, but is very variable, and usually ends approximately 24 hours (0–48 hours) after ovulation (Miro i Roig *et al.*, 2004). Quintero *et al.* (1995) presented the largest percentage of ovulations occurred 24 h (48.1%) and 48 h (37.2%) prior to the end of the period of sexual receptivity behaviour in the mare. Their results showed 12.7% mares with ovulation without behavioural estrus. They found out that „silent heat“ occurs more frequently in May (21.4%) and June (18.1%) (Quintero *et al.*, 1995). These findings agree with ours, as in our study mares with weak estrous signs were observed mainly during the summer months (June, July, August). In this study mares with pathological conditions (silent heat, anovulatory follicle, etc.) were excluded from experiment.

In Slovakia, no studies have been done that evaluate the influence of environmental factors on reproductive parameters in mares. This study is the first which use the exact data from the Slovak Hydrometeorological Institute.

As far as climate change is concerned, the aim was also to find out how these changes influenced reproductive parameters in the mares.

Our results may be helpful in equine breeding in our climatic conditions as the wrong timing in live cover or artificial insemination in mare causes significant financial losses and delay the time the owner obtains an offspring from the mare. Due to long mare's gestation period, it is crucial to determine the optimal time for either live covering or artificial insemination. Repeated insemination and covering of the mare can lead to several problems. A common problem after artificial insemination is emergence of post-service endometritis. This condition has to be treated as it may result in delay of the next covering of the mare. The inappropriate timing of breeding can lead to injury of the mare or the stallion. Many injuries require long therapy which increases the costs for the owner.

The number of horses in Slovakia is still low. Among the Central European countries, Slovakia has the smallest number of horses even though it has increased in past decades thanks to foreign import. On the other hand the number of covered or inseminated mares has decreased. Our results could help breeders and veterinary professionals to increase the number of inseminated mares and therefore increase the number of foals born in Slovakia.

CONCLUSION

Our study, similarly to studies of other authors, proved the significant impact of photoperiodism and ambient temperature on the length of the heat in mare. It was proved that during the breeding season the duration of the heat shortens with elongation of the day light and temperature in-

crease and vice versa. A more detailed survey would require a longer duration and a greater number of mares, as well as research in the surrounding countries with similar climatic conditions.

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