Original Paper

Change in the Size of Pre-Ovulatory Follicles During the Mare Breeding Season

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The mare is a seasonally polyestrous animal whose reproductive activity is regulated by hormonal mechanisms and influenced by environmental factors such as temperature and photoperiod. The aim of this study was to monitor seasonal changes in the size of preovulatory (PO) follicles of mares during the breeding season and to evaluate their relationship to the average daily temperature and the length of the daylight period. The study was carried out on 247 mares of different breeds aged 3–24 years, which were examined between April and September over a five-year period at the Equine Clinic of the University of Veterinary Medicine and Pharmacy in Košice. Follicle development was monitored ultrasonographically, supplemented by the determination of progesterone levels. The average size of PO follicles ranged from 5.00 ± 0.51 cm in April to 4.70 ± 0.42 cm in September, with no significant differences confirmed between individual months ($p = 0.28$). However, a multifactorial analysis based on individual measurements ($n = 247$) demonstrated a significant effect of average daily temperature on follicle size ($p = 0.008$), whereas day length did not show a significant effect ($p = 0.233$). Overall, follicle size showed a decreasing trend during the season and towards the end of the breeding season ovulation occurred at smaller diameters. The results indicate temperature as a key factor influencing seasonal changes in follicle size. These findings have practical importance for veterinarians and reproductive specialists, as reliance on a fixed follicle size threshold may lead to incorrect determination of the time of ovulation and lower insemination success at the end of the season.

Keywords: follicle, mare, photoperiod, reproduction, temperature

1 Introduction

The mare is a seasonally polyestrous animal (Nagy et al., 2000). The mare is a seasonally polyestrous animal (Nagy et al., 2000). However, the character of reproductive seasonality is markedly influenced by geographical location and photoperiod length. In regions close to the equator, where day length remains relatively stable throughout the year and pronounced seasonal fluctuations in the light regime are absent, an almost continuous polyestrous cycle is expected, with mares potentially exhibiting ovarian activity year-round (Osborne, 1966). In contrast, in temperate and subarctic regions, the reproductive cycle is strongly seasonal, with a typical period of winter anestrus followed by a transition to full cyclic ovarian activity in spring. The physiological reproductive, or ovulatory, season occurs from April to September in the Northern Hemisphere (Scott, 2020; Osborne, 1966) and correspondingly from October to April in the Southern Hemisphere (Nolan, 2019; Osborne, 1966).

Understanding the mechanisms of hormonal interaction in mares has a fundamental impact on the economic significance in horse breeding (Kim et al., 2022). According to McKinnon et al. (2011), the regulation of the estrous cycle is ensured through the hypothalamus – pituitary – ovarian axis, which enables the release, transmission, and subsequent action of sex hormones capable of binding to specific receptors and thereby triggering a cascade

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of specific reactions. Follicular development occurs, according to Chavatte and Palmer (1998), in follicular waves. While the secondary wave may occur during diestrus, when follicles often do not reach ovulatory size and undergo regression, the primary follicular wave leads to the growth of the dominant follicle, which ovulates under the influence of increased levels of luteinizing hormone (LH) (Samper et al., 2007). Noakes et al. (2019) state, that follicle-stimulating hormone (FSH) stimulates the growth of multiple follicles, from which one dominant follicle is gradually selected. During the selection of the dominant follicle in mares, one follicle begins to grow more rapidly than the others, resulting in a marked difference in their size and leading to this follicle becoming the ovulatory one. This dominant follicle simultaneously produces estradiol, which decreases FSH concentrations and modulates LH levels in order to maintain its leading position and prevent further growth of subordinate follicles (Ginther, 2023).

In mares with a single follicular wave, the dominant follicle reaches preovulatory size above 30 mm (Brinsko et al., 2011) and after the threshold of estrogen secretion is reached, a larger amount of gonadotropin-releasing hormone (GnRH) is released, followed by LH, which triggers ovulation (Wintzer et al., 1999; Samper et al., 2007). Follicle diameter is used in practice, according to Cuervo-Arango and Newcombe (2008), as a guiding tool to predict ovulation in mares. It is the simplest and probably the most widely used clinical criterion, however, as they themselves state, the wide range of diameters of preovulatory follicles in mares makes this criterion unreliable for estimating the optimal time of breeding. Ovulation is also associated with a change in follicle shape from spherical to pear-shaped (Brinsko et al., 2011) and according to Samper (2000) its size can only be determined by ultrasonographic examination, since he considers the palpation method inaccurate. In addition to ultrasonographic examination, it is also possible to use a diagnostic method based on monitoring progesterone levels in blood, which, in repeated measurements, should show a decreasing trend with a value below 1 ng/ml (Brinsko et al., 2011).

The estrous cycle is regulated not only hormonally but also through the photoperiod (Šťastná and Šťastný, 2016) and environmental temperature (Doležal et al., 2015). A nervous stimulus caused by the action of light on the retina of the eye is transmitted to the pineal gland, where it is transformed into a hormonal output in the form of melatonin (Šťastná and Šťastný, 2016). Its high level, which reaches a peak especially during the winter months, inhibits the release of GnRH, which causes suppression or even complete cessation of reproductive activity during this period (Davies-Morel, 2003). Also according to Samper et al. (2007), during winter anestrus the secretion of GnRH is low, the ovaries show no activity and follicles usually do not exceed a size greater than 10 mm. In spring, the lengthening of the day stimulates the resumption of the cycle and follicle development, although not all follicles reaching preovulatory size actually ovulate (Brinsko et al., 2011). Before the onset of full reproductive activity, some mares may develop 1–3 anovulatory waves characterized by follicles larger than 40 mm, which undergo regression before ovulation itself. The development of these follicles differs from dominant ones in that LH levels are lower than at subsequent ovulations, they grow more slowly although they may reach a larger diameter than a preovulatory follicle (Cuervo-Arango and Clark, 2010). During the period of full reproductive activity in spring and summer, mares exhibit regular cycles with growth and ovulation of dominant follicles (Samper et al., 2007). According to Rua et al. (2019), follicle size should be evaluated daily in order to predict ovulation and the optimal time for insemination.

The aim of our study was to monitor changes in the size of preovulatory follicles of mares during the breeding season in relation to changes in average temperature and day length, and to evaluate their effect on the process of ovulation. We hypothesized that during the season there is a gradual reduction in the size of preovulatory follicles, assuming that ovulation at the end of the season occurs at a smaller follicle diameter than at its beginning.

2 Material and Methods

2.1 Animals

The experiment was carried out on 247 mares of warmblood breeds aged 3–24 years, which were brought to the Equine Clinic of the University of Veterinary Medicine and Pharmacy in Košice over a five-year period. The mares were examined during the breeding season from April to September in all five years. Diagnostics and subsequent examinations took place in the premises of the clinic. The horses were housed throughout the entire period in accordance with the recommended standards for horse welfare defined by the International Equestrian Federation (FEI Code of Conduct for the Welfare of the Horse, 2025). In order to ensure optimal conditions and the safety of the animals as well as the examining and assisting personnel, they were placed in a fixation stocks during the examination.

2.2 Methods

At the beginning, the mares were subjected to adspection and gynecological examination. In mares, we observed the presence of vaginal discharge, frequent urination, reddened and swollen vulva. In case a stallion was available, we monitored willingness to mate. Subsequently, the internal genital organs were examined by rectal probe. Based on the shape and size of the follicles, it was possible to assess and confirm whether the mare was in estrus. On the USG image, we detected a dominant follicle on the ovaries with the assumption that one of them would ovulate. Impending ovulation was indicated by disruption of the integrity and thickening of the follicle wall, the presence of hyperechogenic particles inside the follicle and the size of the endometrial folds of the uterus. We evaluated their edema and the presence of fluid between them. At the initial examination, we collected blood samples to determine progesterone, which should be lower than 1 ng/ml in mares in estrus. Thanks to regular monitoring of mares, we had a very precise picture of the development of the size and shape of follicles. A basic feature of the preovulatory follicle for us was the change of its shape, when it changed from oval to pear-shaped, the size was measured as the largest dimension of the follicle. It was also possible to observe a decrease in follicle tension during transrectal palpation of the ovaries. Ultrasonographic examination (USG) was performed using a SonoScape E1V device (SonoScape Medical Corp., China) with a rectal probe adjustable in the range of 4–8 MHz. Given the nature of the examination, the necessary tools were used (disposable rectal gloves, lubricants), which protected the veterinarian from contact with feces and at the same time protected the rectal mucosa from injury.

In order to determine the level of progesterone, blood samples were taken from the vena jugularis, which were transported and examined in the laboratory RIA laboratory s.r.o. at Kukučínova 84/5 in Košice, Slovakia. Data on average daily temperature and day length for the location where the mares were examined were obtained from publicly available online databases. The average daily temperature was taken from the portal tutiempo.net (https://en.tutiempo.net/), while the data on day length were obtained from the website sunrise-and-sunset.com (https://sunrise-and-sunset.com/). Both databases were set for the geographical location of Košice.

2.3 *Statistical Analyses*

Statistical analysis was carried out using the SAS Enterprise Guide software (SAS Institute Inc., Cary, NC, USA). The measured data were subjected to distribution analysis, which confirmed their normal distribution and therefore they were subsequently analyzed using parametric statistical tests. Methods of summary statistics and regression analysis were applied. The effect of the month of the breeding season on the size of the preovulatory follicle was assessed by one-way analysis of variance and the statistical significance between individual measured values was tested using Tukey's test. The effect of environmental temperature and photoperiod length on the size of the preovulatory follicle was analyzed by multifactorial analysis of variance.

3 Results and Discussion

As shown in Table 1, the average size of preovulatory follicles during the breeding season ranged from 5.00 ±0.51 cm in April to 4.70 ±0.42 cm in September. These values are consistent with the data reported by Cuervo-Arango and Newcombe (2008), according to whom the diameter of preovulatory follicles in mares is in the range of 3.5–5.0 cm, while maximum values may reach up to 6.5–7.0 cm. This wide variation, according to them, is related to several factors, such as the period of breeding, the number of preovulatory follicles, breed affiliation or individual variability. Moreover, according to Gastal et al. (2006), compared to the natural cycle, smaller preovulatory follicles may also appear in cases when ovulation is induced using hCG. A field study by Ginther (1992) showed, that none of the 181 mares ovulated before the follicle diameter reached 3.5 cm. In our group of mares, the average values of preovulatory follicle size were located in the upper part of the published interval. The size of the preovulatory follicle itself is an important, but not always reliable indicator of the optimal time for

Table 1 Seasonal changes in the size of preovulatory (PO) follicles in relation to temperature and day length

Month	Number of examined mares	Average PO follicle size (cm)	Average temperature (°C)	Average photoperiod (h)
April	19	5.00 ±0.51	8.6 ±0.87^{a}	13.66 ±0.01^{a}
May	86	4.87 ±0.46	14.2 ±1.32^{b}	15.21 ±0.01^{b}
June	65	4.93 ±0.49	20.4 ±1.39^{c}	16.02 ±0.00^{c}
July	46	4.76 ±0.47	20.7 ±0.96^{d}	15.62 ±0.01^{d}
August	22	4.85 ±0.48	21.4 ±1.26^{e}	14.24 ±0.01^{e}
September	9	4.70 ±0.42	14.8 ±1.69^{f}	12.33 ±0.60^{f}
p-value		0.28	< .0001	< .0001

Means marked with different indices (a, b, c, d, e, f) show statistically significant differences at the significance level of $p < 0.05$

Table 2 Effect of environmental factors on the size of preovulatory (PO) follicles

Parameter	*p*-value	
Average PO follicle size (*n* = 247)	average temperature	average photoperiod
	0.008	0.233

insemination and according to Brinsko et al. (2011) its use is appropriate to combine with other indicators of reproductive activity, such as progesterone level.

One-way analysis of variance did not demonstrate statistically significant differences in the size of preovulatory follicles with respect to the month of breeding ($p = 0.28$). In contrast, average daily temperature ($p < 0.001$) and photoperiod length ($p < 0.001$) showed statistically significant changes during the breeding season. The lowest average temperature was recorded in April (8.6 °C) and the highest in August (21.4 °C). The average day length ranged from the lowest value of 12.33 h in September to a maximum of 16.02 h in June (Table 1).

From Table 2 it follows that the multifactorial analysis of variance based on individual measurements ($n = 247$) confirmed a significant effect of average daily temperature on the size of PO follicles ($p = 0.008$), whereas day length did not show a significant effect ($p = 0.233$). The overall model was at the threshold of statistical significance ($p = 0.05$).

The seasonal development of the size of preovulatory follicles and the average daily temperature during the breeding season is shown in Figure 1. The average follicle size showed a decreasing trend during the season ($R^2 = 0.714$), while the average daily temperature showed the opposite trend ($R^2 = 0.3142$) with a maximum in August and a decrease in September. The relationship between average temperature and the size of preovulatory follicles was tested separately by multifactorial analysis of variance based on individual measurements ($n = 247$), which confirmed a significant effect of average daily temperature on the size of preovulatory follicles ($p = 0.008$) (Table 2). In the study by Rua et al. (2019), which examined the effect of temperature on the size of the preovulatory follicle, a significant difference was not demonstrated ($p > 0.05$). From Figure 1 it is also evident that the largest follicles occurred at the beginning of the breeding period, while with the gradual increase in temperature they decreased towards the end of the season. This phenomenon can also be explained by physiological changes that take place during the spring transitional period. According to Nagy et al. (2000), the resumption

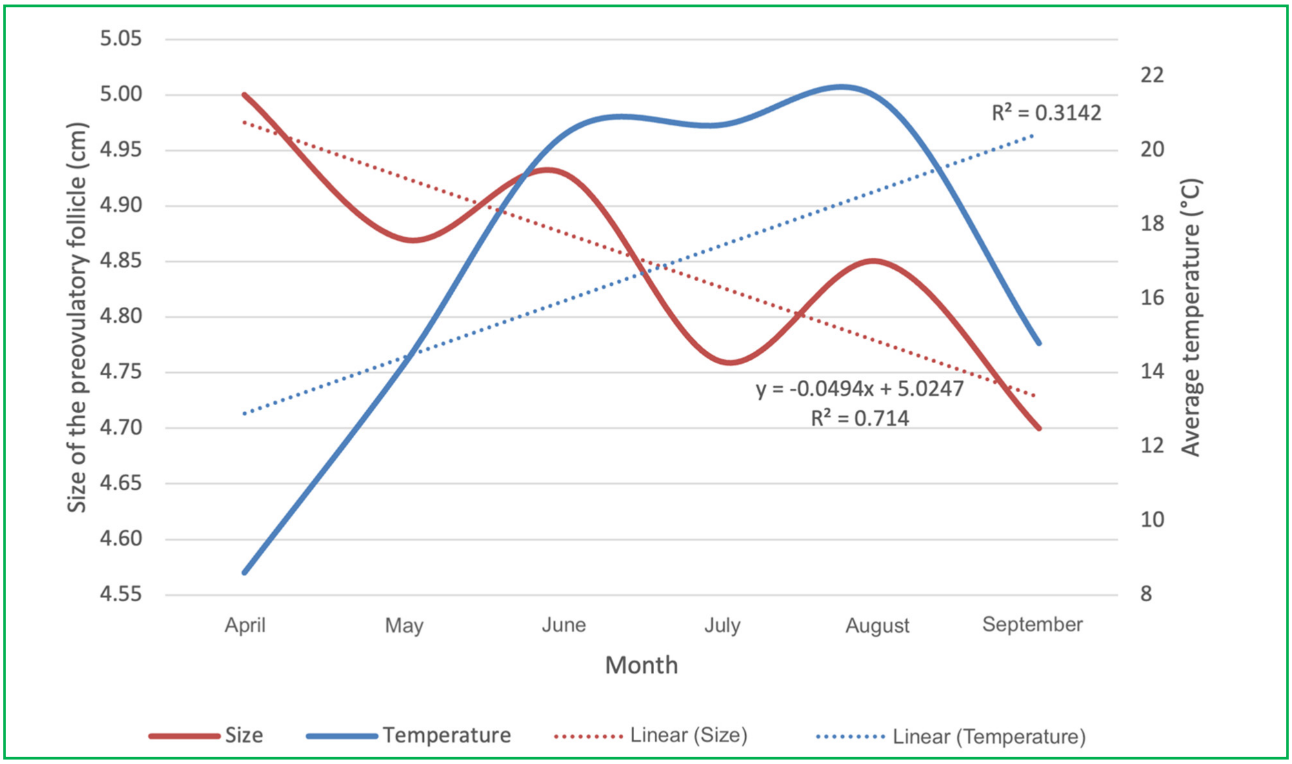

Figure 1 Development of the diameter of preovulatory follicles and average temperature during the breeding season

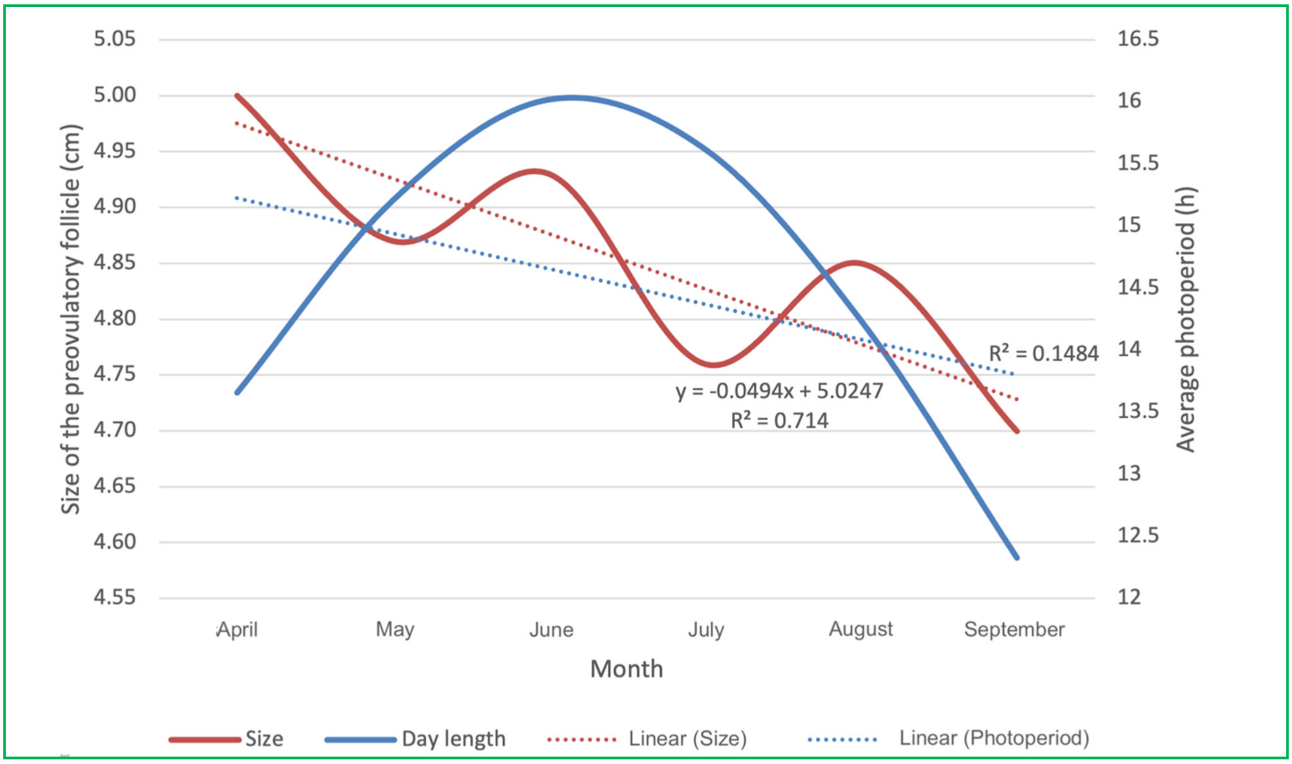

Figure 2 Development of the diameter of preovulatory follicles and average day length during the breeding season

of ovarian activity during this period is associated with a gradual increase in hypothalamic activity, during which the effects of melatonin recede. As the mare progresses through the transitional period, estradiol production in the preovulatory follicle increases, which may reflect the growing size of the follicle. However, our findings are in contrast with the results of Alkhadrawy et al. (2024), who state that follicles ovulated during the hot months were larger than those ovulated during months with lower temperature. This difference may be due to the different breed composition of the studied population or the different management of the mares.

In Figure 2, the development of the average size of preovulatory follicles and the length of the daylight period during the breeding season is shown. The day length increased from April to June, reached a maximum in June (16.02 h) and subsequently gradually decreased until September (R^2 = 0.1484). Although the decrease in follicle size temporally corresponded with the shortening of day length in the second half of the season, the multifactorial analysis of variance based on individual measurements (n = 247) did not demonstrate a statistically significant effect of this factor (p = 0.233). A similar conclusion was reached by Rua et al. (2019), who recorded a larger average follicle size at shorter day length, but this difference was not statistically confirmed ($p > 0.05$).

Our results showed that the size of preovulatory follicles had a decreasing trend during the breeding season with ovulation at the end of the season occurring at a smaller follicle diameter than at its beginning. A similar phenomenon was described by Davies-Morel et al. (2010), according to whom mares bred at the end of the season, especially in the case of multiple ovulations, ovulated more frequently from smaller follicles. The authors also pointed out that a smaller preovulatory diameter may be related to lower oocyte viability, which may manifest itself in reduced fertility of mares during this period. These findings support the need for careful monitoring and reproductive management of mares bred at the end of the season.

4 Conclusions

Although statistically significant differences between the average monthly values of preovulatory follicle size were not confirmed, the systematic evaluation conducted throughout the entire breeding season provided a comprehensive view of their seasonal dynamics and revealed a decreasing trend in follicle size. The results further suggest a significant role of ambient temperature in the dynamics of ovulation under practical breeding conditions. This trend was statistically significantly influenced by average daily temperature, whereas day length had no significant effect on preovulatory follicle size. Our findings therefore support the assumption that environmental conditions, particularly temperature, may contribute to seasonal changes in preovulatory follicle size, which is consistent with the original hypothesis that ovulation at the end of the season occurs at a smaller follicle diameter.

These findings have a significant practical impact for veterinarians and specialists in equine reproduction, as they highlight the need to take seasonal environmental factors into account when determining the optimal time for insemination. If the size of preovulatory follicles decreases towards the end of the season, reliance on a fixed threshold follicle size may lead to an incorrect estimation of the time of ovulation and lower fertilization success.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

Magdaléna Zaťková: Writing original draft, data curation, Investigation; Marko Halo: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision; Eva Mlyneková: Methodology, Project administration, Writing original draft, Supervision; Vladimír Hura: Data curation, Supervision, Writing review editing; Ivan Imrich: Data curation, Writing original draft, Visualization; Stanislav Zaťko: Data curation, Writing review & editing, Resources

AI and AI-Assisted Technologies Use Declaration

No artificial intelligence (AI) or AI-assisted technologies were used during the preparation of the manuscript.

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