

Effect of Age on the Date of First Ovulation in Mares Subjected to an Artificial Lighting Regimen During Seasonal Anestrus

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Abstract

Previous studies have shown that artificial lighting can reduce the duration of anestrus in the horse. Here, we investigated whether age can affect the date of first ovulation in mares subjected to an artificial lighting regimen beginning before the winter solstice. Mares ($n = 224$) of different breeds aged 1 to 27 years were divided into five groups by age: a) 1–5 years ($n = 71$), b) 6–10 years ($n = 38$), c) 11–15 years ($n = 44$), d) 16–20 years ($n = 30$), and e) 21–27 years ($n = 16$). 25 mares apparently failed to respond to treatment and were excluded from the analysis. Light treatment began on a mean starting date of November 27. Ovulation was determined through transrectal ultrasonography at regular intervals. The age of the mare significantly influenced the date of first ovulation, with mares aged 1 to 5 years ovulating significantly earlier than all other age groups ($P < 0.01$). The interval from the start of treatment to ovulation in this group was shorter than for all other groups. A significantly larger proportion of mares in the youngest group (25.7%) ovulated before February 1 compared to all other age groups combined (6.9%). The proportion of mares that failed to respond to treatment was significantly larger in mares older than 15 years ($P < 0.01$). These results show that under winter artificial lighting, first ovulation occurs earlier in young mares, likely due to a lessened entrainment to the natural photoperiod.

Keywords

Circannual rhythms; horse; estrous cycles; ovulation; photoperiod, seasonal breeding

1. Introduction

In temperate climates, mares are seasonal breeders, entering a period of ovarian inactivity with declining daylight. The entry into anestrus and the vernal transition to ovarian cyclicity have been comprehensively described in [1]. However, unlike the spring transitional period that leads to the resumption of ovarian cycles within a defined timeframe [2], the autumn transitional period that leads to anestrus (if the term 'transitional' is applicable here) is variable. Younger mares and those on a reduced caloric balance may have a final ovulation as early as August in the Northern Hemisphere, whereas the majority continue to cycle into winter, ceasing ovarian activity in November or

December [3]. Data from this Clinic showed that occasional mares may cycle into January or even February before entering an anestrus phase until April or, rarely, even May. A small percentage of mares continue to cycle throughout the winter, even under outdoor conditions [4]. At lower latitudes, however, it was found that 55% of 11 mares cycled all winter [5].

The mean date of the first seasonal ovulation at the latitude of this Clinic (53°N), where previous studies were undertaken [4], is April 23rd, with no more than a 4-day variation between seasons (unpublished). In Finland, however, at a latitude of 61°N, only 14% and 62% of barren mares were found to be cyclic by the end of April and May, respectively [6]. They

noted that 3-year-old mares in work ovulated earlier than the barren mares, with 26% and 97% of 3-year-old mares cyclic by the end of April and May, respectively, compared with 14% and 68% of the barren mares. This difference was attributed to differences in feeding, exercise, and stable lighting rather than age. It was also observed that mares younger than 5 years stopped cycling earlier than older mares [7], and that mares older than 15 years took longer to begin cyclic ovarian activity. At our clinic, a previous study showed that all four 3-year-old mares had stopped cycling by the end of September, compared with only 3 of the 17 older (4- to 25-year-old) mares [4].

It was originally reported that exposure to artificial lighting during late winter or early spring significantly reduced the duration of anestrus in mares [8], and subsequent studies revealed that extending the light phase of the 24-hour light:dark cycle from 12 hours to 14.5–16 hours a day was effective in hastening the onset of ovarian cyclicity [9–13]. Thus, this light treatment has been widely used in our clinic to enable early conception. Since earlier publications had suggested that the best effect could be obtained with a lighting regimen started at the end of December or early January (Northern Hemisphere), this strategy was adopted for years. Later, however, we found that ovulation could be advanced further by starting an earlier light regimen. During the analysis of data recorded over many seasons, it was noticed that this advancement was even more effective in young mares. This study was therefore undertaken to determine whether age affects the time of first ovulation in mares subjected to an artificial lighting regimen during seasonal anestrus.

2. Materials and Methods

Seasonally anestrous mares, used both for breeding and as embryo transfer recipients, were studied to determine the effect of age on the date of the first ovulation following treatment with artificial lighting in an outdoor arena equipped with floodlights that imparted a light intensity of approximately 300 lux at the level of the horse's head. Daylength was extended to at least 16 hours, beginning at dusk and ending at midnight. The data were gathered from 32 seasons of observations.

Mares ($n = 224$) aged 1 to 27 years (mean = 12 years), including Irish Draught and other breeds and crossbreeds, were maintained at pasture until the beginning of light treatment, at which point they were corralled in the arena under 16 hours of light. The large majority of mares had been wintered under ambient light in the years prior to treatment. Mares were fed *ad libitum* baled haylage in the arena and at pasture when grass became scarce. All mares were in good body condition (scores 5–8) from the beginning of treatment until ovulation. Photoperiodic conditions were the same each season.

Mares were examined per rectum with ultrasound at regular intervals during the autumn transition into anestrus to determine the final ovulation of the breeding season. Mares that continued to ovulate after the beginning of light treatment were excluded from the study if the interovulatory interval between the last ovulation and the first ovulation of the next season was less than 60 days. Anestrous mares were examined every 2–3 weeks, then with increasing frequency,

from every 4–5 days to daily, and finally three times daily as follicle diameters and edema scores increased preceding the first ovulation. Some young mares were already in deep anestrus when first examined prior to treatment, and therefore, the date of the last ovulation was not known; otherwise, the date of ovulation was determined to within 24 hours. All ovulations were spontaneous. No mare was given any ovulation-inducing treatment.

Most mares ($n = 199$) first ovulated following treatment between December 16 and March 31. Mares that ovulated later than March 31 ($n = 25$; 11%) were deemed to have failed to respond to the lighting regimen and were excluded from the analysis.

For analysis, mares were divided into five groups by age: a) 1–5 years ($n = 71$), b) 6–10 years ($n = 38$), c) 11–15 years ($n = 44$), d) 16–20 years ($n = 30$), and e) 21–27 years ($n = 16$). Light treatment began on a mean date of November 27 (range: November 17 to December 11). The individual mean dates for the start of light treatment for each age group were November 25, November 26, November 29, November 28, and November 24 for age groups a–e, respectively.

2.1. Statistical Analysis

Continuous data (e.g., the interval between February 1 and the first ovulation of the year) were tested for normality using the Anderson-Darling test. As the data were not normally distributed, the effect of mare age on the day of the first ovulation was determined using the non-parametric Kruskal-Wallis test, followed by the Mann-Whitney test for pairwise comparisons.

It is important to note that although we acknowledge that a multifactorial analysis could have been employed to account for possible effects of individual mares, a given year, etc., the main objective of the study was specifically to determine the overall effect of mare age on the variables of interest. Categorical data (e.g., the proportion of mares ovulating before February 1) were examined using the Chi-square test.

3. Results

The mean date of the start of treatment (November 25, 26, 29, 28, and 24) did not differ between age groups (Table 1).

The median interval from February 1 to the first ovulation of the year was influenced by mare age group ($P < 0.001$). The youngest group (mares aged 1–5 years) ovulated earlier than any of the other age groups ($P < 0.001$). The median interval between mares aged 6–10 years (16.5 days) and those older than 15 years (19.5 days) was significantly different ($P = 0.029$). The individual median intervals from February 1 to the first ovulation were 8.5, 16.5, 15.5, 19.5, and 19.5 days for age groups 1–5, 6–10, 11–15, 16–20, and 21–27 years, respectively.

The mean interval from the start of treatment to ovulation for the youngest group (75 days) was shorter than for all other groups (mean interval range for the other age groups: 81–88 days) (Table 1).

A significantly ($P < 0.01$) larger proportion of mares in the youngest group (25.7%) ovulated in December or January (before February 1) compared with all other age groups combined (6.9%) (Figure 1).

Table 1: Mean dates for the start of artificial light treatment and first ovulation, time (days) elapsing between the start of light treatment and first ovulation, and the number and proportion of mares ovulating before February 1 in groups of mares aged: a) 1 to 5, b) 6 to 10, c) 11 to 15, d), 16 to 20, and e) 21 to 27 years.

Age Group	a	b	c	d	e	Failed to respond
Age (years)	1–5	6–10	11–15	16–20	21–27	
n	70	38	44	31	16	25
Start date of long-day treatment	25.11	26.11	29.11	28.11	24.11	26.11
Range of dates	17.11–3.12	9.11–4.12	21.11–7.12	23.11–9.12	8.11–11.12	1.11–10.12
Mean date of first ovulation	9.2*	21.2	19.2	21.2	20.2	24.4**
Range of dates between individuals	16.12–28.3	20.1–29.3	25.1–30.3	27.1–21.3	13.1–28.3	
Start of long-day treatment to ovulation (days)	75*	87	81	85	88	146
Range within groups (days)	67–83	69–94	72–95	79–91	61–104	
Number of mares that ovulated before February ^{1st}	18*	1	5	1	2	
% of mares that ovulated before February ^{1st}	25.7*%	2.6%	11.4%	3.2%	11.8%	

* $P < 0.001$ for mares aged 1–5 compared to all other groups; ** $P < 0.001$ for mares that failed to respond to light treatment compared to mares that did respond.

The mean start date of treatment for mares that failed to respond ($n = 25$) was not different (November 26) (Table 1). The mean date of first ovulation in these mares (April 27) was significantly different from that of all other groups (February 16). The mean age of outliers was not different (12.6 years) but was not evenly distributed; among mares aged 16 to 27 years, 25.5% were outliers, compared with 8.6% in mares aged 1 to 15 years.

Had the outliers been included in the analysis, the mean ovulation date for mares aged 1 to 5 years (February 14) would have been significantly different from all other age groups (February 25.4, February 23.6, March 5.0, and March 5.3 for groups b to e, respectively).

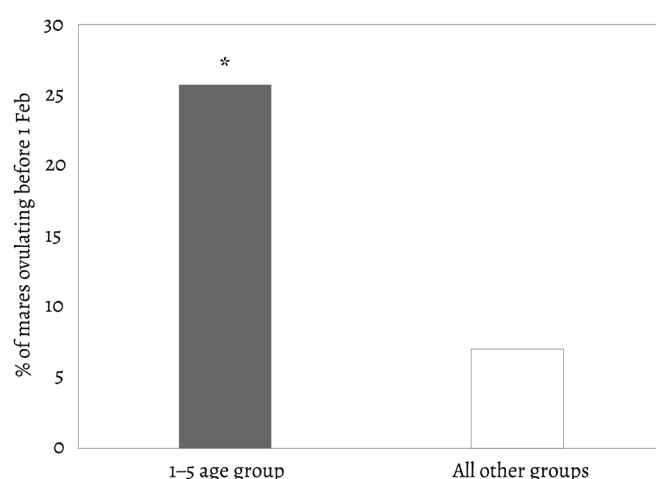


Figure 1: Effect of age on the proportion of mares ovulating before February 1 in mares subjected to an artificial lighting regimen designed to increase the duration of the light phase of the 24-hour light:dark cycle to 16 hours per day during seasonal anestrus. The average start date of light treatment was November 27; lights were turned on at the beginning of dusk and off at midnight. * $P < 0.001$ for mares aged 1 to 5 years compared to all other age groups combined (6 to 27 years).

4. Discussion

The results of this study show that the duration of seasonal anestrus in mares subjected to an artificial lighting regimen during the winter is significantly affected by age and reveal that, under these experimental conditions, first ovulation occurred earlier in young mares (1–5 years) compared with their older counterparts. This study was not undertaken to investigate the effects of artificial lighting on the duration of seasonal anestrus per se, as this effect is already well established [1,3,8–13].

In the current investigation, the mean date of the first ovulation in 3-year-old mares was February 9.7th, and for 1- to 5-year-olds, it was February 8.1th. Mares older than five ovulated, on average, about 11–12 days later (February 20th). These results are in agreement with previously reported findings in Finn Horses [14], where 3-year-old mares subjected to artificial lighting were shown to exhibit an earlier onset of seasonal activity than older mares (6 to 14 years). In that study, 67% ($n = 8$) of 3-year-old mares were cyclic by the second week of February, compared with only 13% ($n = 2$) of mares in the older group (>3 years), whereas by the fourth week, 92% of 3-year-old mares were cyclic compared with 63% of older mares. In our study, the mean dates for 3-year-old and older mares were February 9th and February 20th, respectively, which are comparable to the previously reported dates of February 13th and February 23rd for similar age groups [14]. Both studies show that, when subjected to an artificial lighting regimen designed to increase the duration of the light phase of the 24-hour cycle during the winter, mares under the age of six ovulate about 10 to 12 days earlier than older mares. In our study, there was also a difference between mares aged 6 to 10 years and all mares older than 15 years.

In the current study, the majority of mares had previously been kept at grass under ambient light conditions during most winters. In a related study [14], no data were provided regarding whether mares had been exposed to

ambient or artificial light in prior seasons. However, it was confirmed that although those mares were housed, they had never been subjected to an artificial lighting regimen in previous winters (T. Katilla, personal communication). In both studies, therefore, it appears that most mares had been wintered under ambient light conditions for several years. It is possible that older mares were more strongly entrained to ambient light conditions, which regulate the timing of the first ovulation of the year. At our clinic, this occurs consistently around April 23rd. It appears that this entrainment may have, to some extent, impaired the response to the increase in daylength that began around two months earlier.

We have previously shown that horses are extremely sensitive to light cues and that the influence of photoperiodic signals on circadian biological rhythms in this species is different from the way in which light regulates their circannual physiological cycles [15]. Indeed, we have shown that whereas horses display weak circadian rhythms that are masked by the 24-hour light:dark cycle, thus allowing a rapid resetting following a photoperiodic phase shift [16], they possess extremely strong circannual rhythms which are entrained to a 12-month cycle by the prevailing photoperiod. This is a strategic mechanism of adaptation to the environment which, to some extent, relates to the different anatomical basis of the circadian and circannual clocks and the way light signals control them [17,18]. Indeed, whereas the circadian master clock is located in the suprachiasmatic nucleus of the hypothalamus and is entrained by the 24-hour light:dark cycle mainly, although not exclusively, through a monosynaptic pathway from the retina [19,20], the circannual master clock resides in the pars tuberalis of the pituitary gland and is primarily entrained to a 12-month cycle by the nocturnal secretion of melatonin from the pineal gland [21–24]. Due to the robustness of circannual biological rhythms in this species (e.g., reproduction, hair growth, metabolism), resetting a rhythm once it has been entrained by the local photoperiod is difficult to attain and can take up to a full year (D.J. Tortonese, unpublished observations). Thus, the finding in the current study that, under an extended light phase during winter, the first ovulation after anestrus occurred significantly earlier in young mares can be attributed to a reduced robustness of their circannual cycles. This contrasts with the older mares, who had been exposed to the natural photoperiod for many more circannual cycles and had fully adapted their annual reproductive physiology to the local 24-hour light:dark cycle. As several mares in this investigation were assessed across a number of years, further studies will be able to ascertain a putative increase in the robustness of circannual cycles over time, as well as possible effects of individual mares, specific years, and their interactions on the first ovulation after anestrus.

5. Conclusion

Young mares subjected to artificial lighting were found to ovulate significantly earlier than all other age groups. These findings are attributed to a reduced robustness in the circannual cycles of young mares.

Authors' Contributions

J.R.N. collected and documented the data. D.J.T. helped analyze the data and write the paper, including the statistics and discussion.

Data Availability

The data supporting the findings of this study are available upon request from the corresponding author.

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Conflicts of Interest

No conflicts of interest to be disclosed

Ethical Approval

Ethical approval was not required since there are no experimental procedures.

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