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## RESEARCH ARTICLE



# Plasma melatonin concentration during the early post-partum period in Thoroughbred mares and their foals

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## ABSTRACT

The authors aimed to determine the plasma melatonin concentration in mares and their new-born foals in the early post-partum period. Blood samples were collected from the jugular vein of 53 mare-foal pairs within twelve hours after parturition. Plasma melatonin levels were measured by ELISA. The melatonin concentration, adjusted for the moment of parturition using a generalised linear model, was 34.58 pg mL<sup>-1</sup> in mares. It was significantly lower (27.63 pg mL<sup>-1</sup>) in the new-born foals. However, the melatonin concentration declined differently by the end of the twelve hours, it decreased less in the offspring than in the mothers. An artificial light supplementation at the end of gestation reduced the melatonin concentration both in mares and their foals by about 10 pg mL<sup>-1</sup>, compared to the controls. An elevated melatonin production may be related to preparation of mares for parturition and ensures the chances of survival of offspring, therefore the melatonin may reach its peak at the moment of foaling regardless of its actual time. The effect of low melatonin concentration in new-born foals might be associated with the foal's health and subsequent performance. The need to monitor the melatonin concentration in the offspring justifies further studies.

## KEYWORDS

plasma melatonin concentration, horse, supplemental lighting, circadian rhythm, post-partum period, foal

## INTRODUCTION

Breeding cycles in horses are influenced by daylight, environmental temperature and nutritional intake level. On one hand, the main trigger is the increasing daylight in spring which results in a lower mean melatonin concentration and re-tunes the hypothalamic-pituitary-ovary axis (HPOA) (Nolan et al., 2017). On the other hand, prolonged darkness inhibits the HPOA. During the anoestrus period, the production of the hypothalamic GnRH and pituitary LH is low (Hart et al., 1984; Sharp et al., 1987). In autumn, the increasing melatonin concentration helps to maintain anoestrus and prepare for the forthcoming onset of cyclic ovarian activity (Sharp et al., 1979; Grubbaugh et al., 1982; Kilmer et al., 1982). In mares, the mean melatonin concentration does not change significantly during the follicular (24.66 pg mL<sup>-1</sup> ± 2.47 SEM) and luteal (23.28 pg mL<sup>-1</sup> ± 1.96 SEM) phases (Altinsaat et al., 2009). In the Northern hemisphere, the melatonin

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concentrations are the lowest in June (15 h light: 9 h dark) at  $23.52 \text{ pg mL}^{-1} \pm 1.24 \text{ SEM}$  and the highest in December (9 h light: 15 h dark) at  $42.41 \text{ pg mL}^{-1} \pm 1.59 \text{ SEM}$  (Altinsaat et al., 2009). Field experience shows that the highest natural fertility coincides with the longest daylight times in mid-summer.

During summer, melatonin administration may cause anoestrus in horses without additional exogenous steroids (Ovid et al., 2018). This kind of intervention prevents interference of oestrus with shows, races and work. Intravenous administration of melatonin during pro-oestrus has been described to decrease the concentration of oestradiol (E2) and progesterone (P4) in the following luteal phase (Cleaver et al., 1993). A four-week-long slow-release melatonin implant (20 mg) has significantly elevated the daylight melatonin concentrations during the breeding season, resulting in  $76.51 \text{ pg mL}^{-1} \pm 34.29 \text{ SEM}$  vs.  $9.97 \text{ pg mL}^{-1} \pm 30.05 \text{ SEM}$ , melatonin implant and placebo, respectively (Peltier et al., 1998).

Melatonin is a peptide hormone with a molecular weight of 232.2 Da. The effect of this hormone in the mammalian organism is complex, including the regulation of the circadian rhythm and the functioning of the pigment cells. Melatonin is one of the regulators of the circadian and annual cyclic events, playing an essential role in the reproductive processes. Melatonin is secreted by the pineal gland (epiphysis) during the dark hours of the day and is considered one of the main hormonal messengers, through which external light information communicates with the whole body. Recent studies have attributed various roles to melatonin in the timing of several physiological processes, including cell metabolism. Classically, however, it is known for its function of regulating the reproductive cycle. It is considered a chemical messenger and is a messenger of the light/dark alternation in the environment synchronizing the circadian rhythms. Its circadian periodicity may be understood as a coordinating signal for other biological rhythmicity or as an endogenous synchronizer (Carcangiu et al., 2018; Giannetto et al., 2020).

Melatonin modulates the sexual development and reproductive processes by activating the HPOA. The neuronal pathway of hormone action is as follows: melanopsin in retina - optic nerve - retinohypothalamic tracts - cervical cranial ganglion. In long-day mammals like horses, melatonin production in response to light deprivation leads to a lack of hypothalamic GnRH and pituitary FSH and LH production. In the horse, the manifestation of the sexual cycle is inhibited by melatonin (Talpur et al., 2018).

Under natural light conditions, the blood concentrations of melatonin in horses show a characteristic circadian rhythm (Guerin et al., 1995). When horses are kept in darkness for 24 h, their melatonin hormone concentration does not show significant variation, because the circadian rhythm is not exclusively maintained by melatonin (see the role of period and timeless genes). Consequently, melatonin level alone, unlike cortisol concentration, is not sufficient to characterize the circadian events (Murphy et al., 2011). Under natural conditions, the melatonin production and

inhibition in horses are controlled predominantly by light and not by an endogenous mechanism (Piccione et al., 2013).

Artificial light stronger than 3 lux may disturb the normal night-time melatonin hormone synthesis. No difference has been found in the inhibition of melatonin production when compared after blue light applied in just one or both eyes (Walsh et al., 2013). Light program can be used to lengthen perceived daylight (Murphy et al., 2019). The early onset of the breeding season in mares can be facilitated by a more extended photoperiod (Burkhardt, 1947). The supplementary artificial light of proper wavelength during winter can induce early ovarian activation and ovulation in barren mares allowing horse breeders to meet the time constraints imposed by gallop racing (Nagy et al., 2000). Similarly, the breeding season may be prolonged by artificially longer daily photoperiod, whether illumination is added before or after the natural daylight (Kooistra et al., 1975). However, data concerning the periparturient melatonin level of the mare and her foal are sparse.

Since there is insufficient data available on domestic animals, there is a need to briefly overview melatonin production in human, especially in the perinatal period. In human, melatonin and its metabolites protect the mother-placenta-foetus connection by stimulating the antioxidant enzyme activity (Reiter et al., 2009). Maternal melatonin has a key role in the development of the foetal central nervous system. By the inhibition of NO-synthase and cyclooxygenase, melatonin has an anti-inflammatory effect. It plays a crucial role in the healthy development of the placenta. The latter not only has melatonin receptors but also produces this compound, assuring placental homeostasis (Reiter et al., 2013). There is a strong relationship between the blood concentration of melatonin and oxytocin during pregnancy and parturition (Kivelä et al., 1991). During pregnancy, the melatonin concentration increases, especially after week 24, and achieves its zenith before the parturition. The labour starts late at night or early morning, when there is a melatonin peak (Cagnacci et al., 1998).

The gene expression of melatonin in uterine cells shows a circadian rhythm. Melatonin is the endogenous trigger of labour. It intensifies the oxytocin-induced uterine contractions through the protein kinase C (Mårtensson et al., 1996) and makes the myometrium sensitive to oxytocin (Karpovitch et al., 2018). At the same time, through  $\gamma$ -hydroxybutyrate, melatonin1, melatonin2 and  $\mu$ -opioid receptors, it has an anxiolytic and analgesic effect (Wilhelmsen et al., 2011). Artificial light decreases the peak concentration of melatonin, thus diminishing the incidence of night parturitions (Olcese et al., 2013).

In human, it is known that melatonin concentration increases as pregnancy progresses. No studies have yet been published on accurately determined concentration of melatonin during gestation or peripuerperal period in the domestic animal species, including the horse. Our hypothesis is that melatonin concentration in mares during the early postpartum period, and its concentration in foals show similar values due to the maternal-foetal-placental connection.



Our second hypothesis is that a long-term supplemental lighting during the day affects the intensity of melatonin production at night. This study was designed to measure the plasma concentration of melatonin in the mare and foal in the hours immediately following the parturition taking into account the impact of various factors that may influence it. From the obtained values, it becomes possible to learn about the trends characteristic of this period, and based on them to create adjustments for certain times.

## MATERIAL AND METHODS

### Study design

Fifty-three pregnant Thoroughbred mares (*Equus caballus*) and their 53 foals were used in this study, which was conducted at a commercial Thoroughbred breeding stud at latitude 53° N from 9th January to 30th April in 2018. The natural day length was 7 h and 50 min at the beginning, and 14 h and 53 min at the end of the study.

All mares were kept on pasture during the day, where they grazed *ad libitum* until 16:00 h, and then in individual stalls at night during the study period. Mares had *ad libitum* access to meadow hay during stall confinement. Additionally, commercially available Red Mills Stud Cubes feed mix (14% crude protein, 4.5% ether extract, crude fibre 10%) was provided: 1 kg mornings, and 2–3 kg in the evenings. Free access to water was available.

### Sampling and determination of melatonin concentration

Ten-ml blood samples were collected from the jugular vein of mares and foals into heparinized vacutainers after parturition. The mares gave birth to the foals at night, between 19:00 and 05:00. At the same time, the blood sampling took place also continuously from 19:30 to 09:30. All parturitions happened in natural darkness, since dawn occurred between 7:20 and 5:20 and sunrise between 8:00 and 6:00 within the study period.

The melatonin production in the horse is acutely sensitive to the horse's exposure to ambient light, and the night-time increase in its values is absent if the sample is taken with white stable lights turned on (Fitzgerald and Schmidt, 1995). For this reason, the samples were taken within a consistent short time interval using a red lamp or dim red light provided by the Equilume Stable Light System (Luminaire) in natural darkness (<1 lux) or semi-darkness following the conditions for external natural lighting in the barn (which means that the animals were subjected to a limited natural photoperiod approximately in the last 3 h of the 12-h sampling period). For the time of sampling, the mares were restrained with a halter and the foals held by hand. The samplings preceded the normal post-foaling veterinary examinations carried out under the light.

The blood samples of individuals chosen for the research came from the samples annually taken in the course of routine veterinary procedures to detect infectious disease

(Equine Infectious Anaemia) and to assess the health status of the foals (Austin, 2013; Pirrone et al., 2014) by a prior agreement, which included sampling close to the time of foaling overnight too. The further use of samples obtained during clinical veterinary procedures for research purposes is not considered an animal experiment under EU directive (Directive 2010/63/EU), so no ethical permit was required.

The samples were kept at 5 °C until centrifugation at 2,000×g for 10 min. The plasma was harvested into Eppendorf tubes and immediately placed in a freezer (−20 °C) for storage. The concentration of melatonin was determined by a universal Melatonin ELISA Kit (ab285251, Abcam plc, Cambridge, UK; analytical sensitivity 4.7 pg mL<sup>−1</sup>; range 7.8–500 pg mL<sup>−1</sup>; intra-assay precision <5%; inter-assay CV <13%), used according to the manufacturer's instructions. Each sample was measured in two parallels, and their mean value is considered as an individual result.

The melatonin test results from mares and foals were pooled. A normality test for the joint data was then performed. This test confirmed the normal distribution of melatonin data (Shapiro-Wilk  $W = 0.98369$ ,  $P = 0.12785$ , Kolmogorov-Smirnov  $d = 0.06587$ ,  $P > 0.20$ ; Lilliefors  $P < 0.20$ ).

Additionally, the quality of presuckle colostrum was determined in Brix % using a refractometer to estimate the colostral IgG content at 20 °C on farm. The colostrum specific gravity (Brix %) also followed a normal distribution (Shapiro-Wilk  $W = 0.96890$ ,  $P = 0.16418$ ; Kolmogorov-Smirnov  $d = 0.10321$ ,  $P > 0.20$ ; Lilliefors  $P < 0.20$ ).

### Background data set

For data processing, the following variables were recorded and calculated:

- *Gestation length*; The number of days from the date of the last service to the parturition,
- *Foal's birth weight*; Measured within 12 h post-partum (pp) using an electronic equine scale, in kg,
- *Foal's sex*; Genital sex of the new-born animal: colt or filly,
- *Mare's weight*; Estimated within a month prior to foaling, in kg,
- *Mare's age*; At foaling, in years,
- *Days from the winter solstice to foaling*; The number of days between 21st December of the previous year and the date of current foaling,
- *Hour of foaling*; Based on accurate knowledge of the moment of foaling, data of hours of birth are sorted into consecutive two-hour time frames for statistics, such as 19:00–21:00, 21:00–23:00, etc.,
- *Minutes from foaling to blood sampling*; The period between exact foaling and blood sample taking, which lasted from 10 to 720 min,
- *Generation*; Categories of animals in mare-foal connection: mares or foals,
- *Treatment*; Light treatment group,  $n = 28$  mare-foal pairs; control group,  $n = 25$  mare-foal pairs.

For the light-treated animals, each stall was fitted with a Luminaire Equilume Stable Light System. This fixture



completed the natural daylight up to 15 h per day (which corresponded to the length of the natural day characteristic of the end of the experiment) with a high-intensity blue component (468 nm, 80 lux). Supplementary illumination of treatment mares began 41 days before the calculated date of foaling, with the aim of re-breeding mares sooner after. The foals were coded according to their mother's lighting group.

## Data analysis

For calculation of tendencies in melatonin concentration, the general linear model (GLM) method was used (Statistica version 14., TIBCO Software Inc. (2020), Palo Alto, CA, USA) with the following fixed variables: *foal's sex*, *hours of foaling* (nested in *generation*), *generation*, and *treatment*. The *gestation length*, *foal's birth weight*, *mare's weight*, and *mare's age*, and *days from the winter solstice to foaling* (nested in *treatment*), as well as *minutes from foaling to blood sampling* (nested in *generation* and *group of mares*), were added as covariates into this model. Data connections in our linear model were estimated by backward elimination, deselecting less relevant parameters. The *P*-value associated with each tested parameter, the least squares mean (LS Mean), and the standard deviation (SD) are listed according

to the analysis. The plasma melatonin concentration was adjusted for the moment of parturition and postpartum hour 6 applying a covariate adjustment methodology. The melatonin concentrations as a function of *hours of foaling* (with 95% confidence intervals) and *minutes from foaling to blood sampling* are demonstrated graphically. The colostrum specific gravity was evaluated with the same GLM (except for *generation*, which was not incorporated).

## RESULTS

Table 1 shows all the parameters included in the regression model, as well as their calculated indicators. The factor that had the least significant impact on melatonin concentration was the *mare's age* ( $P = 0.862$ ). Thus, it was eliminated, and the linear model was refitted. At each subsequent step, the least significant variable was eliminated from the model until all the remaining indicators had *P*-values smaller than 0.05.

The impact of *foal's birth weight* and *gestation length* had relatively high *P*-values (0.727 and 0.643, respectively), indicating that they and melatonin concentration are largely or fully independent variables.

Table 1. *P*-values of regression to plasma melatonin concentrations and statistics of the listed variables (initial intercept of the model:  $P = 0.847$ ; final intercept:  $P < 0.001$ )

Variable	Order of elimination	<i>P</i> -value	LS Mean
Mare's age	1	0.862	8.3 years
Foal's birth weight	2	0.727	54.5 kg
Gestation length	3	0.643	351.6 days
Days from the winter solstice to foaling (nested in treatment)	4	0.350	85.6 days
light-treated		0.920	
control		0.179	
Mare's weight	5	0.295	590.5 kg
Foal's sex	6	0.161	colts: 20.5 pg mL <sup>-1</sup> fillies: 23.1 pg mL <sup>-1</sup>
Hours of foaling (nested in treatment)	7	0.108	23:50 h
light-treated 19:00-21:00		0.443	22.1 pg mL <sup>-1</sup>
light-treated 21:00-23:00		0.305	15.4 pg mL <sup>-1</sup>
light-treated 23:00-01:00		0.220	23.4 pg mL <sup>-1</sup>
light-treated 01:00-03:00		0.453	16.2 pg mL <sup>-1</sup>
light-treated 03:00-05:00		reference	19.0 pg mL <sup>-1</sup>
control-19:00-21:00		0.278	27.0 pg mL <sup>-1</sup>
control-21:00-23:00		0.289	27.2 pg mL <sup>-1</sup>
control-23:00-01:00		0.915	22.3 pg mL <sup>-1</sup>
control-01:00-03:00		0.551	25.4 pg mL <sup>-1</sup>
control-03:00-05:00		reference	22.7 pg mL <sup>-1</sup>
Minutes from foaling to blood sampling (nested in treatment)	not removed	0.038	326.4 min see Fig. 2
light-treated		0.023	
control		<0.001	
Minutes from foaling to blood sampling (nested in generation)	not removed	0.020	326.4 min see Fig. 3
mares		<0.001	
foals		<0.001	
Generation	not removed	0.009	see Table 2
Treatment	not removed	<0.001	see Table 2





The effect of the variables *days from the winter solstice to foaling* (nested in *treatment*), *mare's weight*, and *foal's sex* on melatonin concentration were also negligible ( $P = 0.350$ ,  $0.295$ , and  $0.161$ , respectively).

The effect of *hours of foaling* (nested in *treatment*) on melatonin concentration was rather close to statistical significance. However, its  $P$ -value remained still above  $0.05$ . It was eliminated last from the regression model ( $P = 0.108$ ),

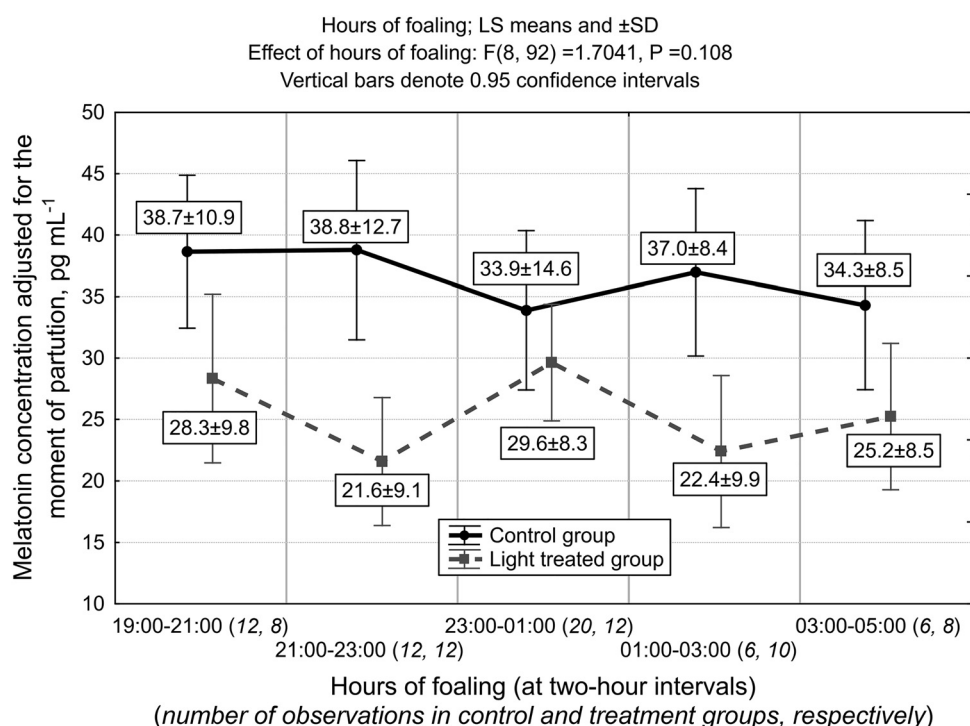


Fig. 1. Distribution of the melatonin concentration adjusted for the moment of parturition according to the hours of foaling. Results are sorted into two-hour-long consecutive time frames around midnight

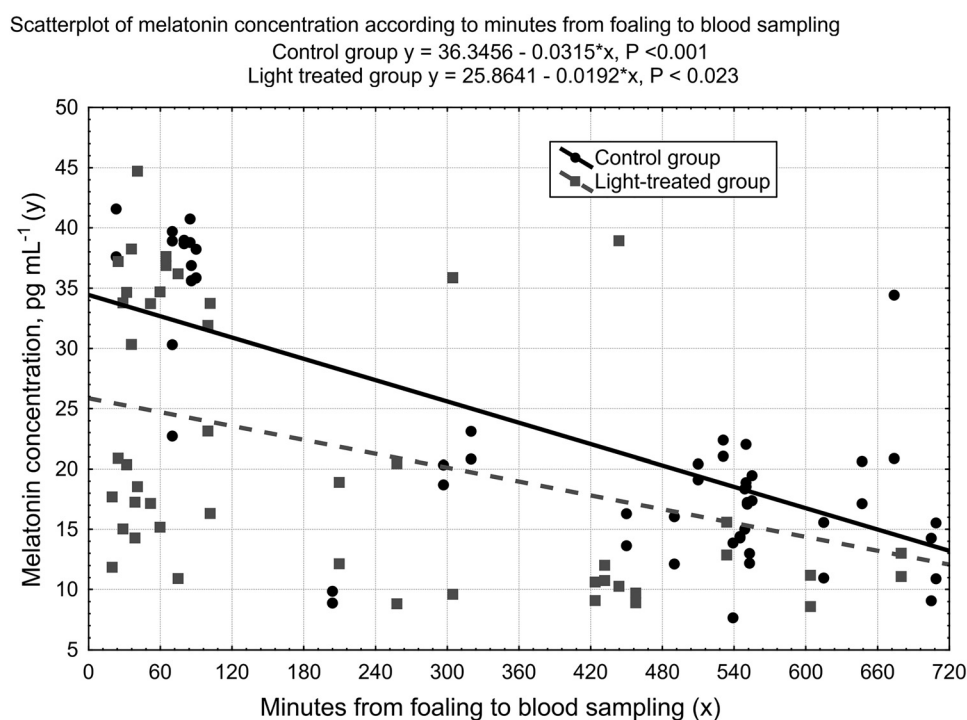


Fig. 2. Scatterplot of melatonin concentration as a function of *minutes from foaling to blood sampling* according to the *treatment*

as shown in Table 1. Additionally, the distribution of melatonin concentration adjusted for the moment of parturition according to the *hours of foaling* is presented in Fig. 1. It can be seen that neither the control nor the light-supplemented group's values show any verified changes during the examined night period.

Blood samples were taken an average of 326.4 min after foaling. The *minutes from foaling to blood sampling* predict the melatonin concentration to a significantly different extent according to the classes of the *treatment* ( $P = 0.038$ ) and *generation* ( $P = 0.020$ ). The alteration of melatonin concentration in the two classes of *treatment* according to the *minutes from foaling to blood sampling* is presented in Fig. 2. The initial values in the control group were higher than those in the light-supplemented group. However, with time change, they decreased in both groups, reaching almost the same level by the end of the 12 h.

The distribution of melatonin concentration in the different *generations* as a function of *minutes from foaling to blood sampling* is shown in Fig. 3. In generations, there was a notable change ( $P < 0.001$ , uniformly) in melatonin concentration concerning the time of taking of blood sample. However, melatonin concentrations did not decrease as strongly in the offspring as in the mothers.

The effect of *generation* and light supplementation on animals (*treatment*) proved to be statistically significant ( $P = 0.009$  and  $<0.001$ , respectively; Table 2). Plasma melatonin reached a remarkably lower concentration in the foal generation and animals with light treatment (20.85 and 18.96 pg mL<sup>-1</sup>, respectively) than that in the maternal generation and control animals (22.86 and 24.75 pg mL<sup>-1</sup>,

Table 2. The difference in adjusted plasma melatonin concentrations according to the fixed effect of *generation* and *treatment* (where *minutes from foaling to blood sampling* was the regressor, *n* refers to the number of observations)

		Plasma melatonin concentration adjusted for the moment of parturition, pg mL <sup>-1</sup>		Plasma melatonin concentration adjusted for postpartum hour 6, pg mL <sup>-1</sup>	
Fixed effect	<i>n</i>	LS Mean and SD		LS Mean and SD	
<i>Generation</i> ( <i>P</i> = 0.009):					
mares	53	34.58	13.77	22.86	8.25
foals	53	27.63	12.43	20.85	8.20
<i>Treatment</i> ( <i>P</i> < 0.001):					
light-treated	56	25.86	11.79	18.96	8.76
control	50	36.35	16.93	24.75	8.68

respectively) if we take into account values adjusted for the 6th hour after foaling. These can also be considered as values obtained close on the average of the covariate (326.4 min). The melatonin concentration values, and at the same time the difference between them, became higher when the comparison was made by adjusting to the moment of parturition (27.63 vs. 34.58 pg mL<sup>-1</sup> and 25.86 vs. 36.35 pg mL<sup>-1</sup>, respectively). None of the effects tested, including treatment ( $P = 0.455$ ), showed a significant relationship with colostrum specific gravity. The mean Brix % of the whole investigated mare population was 23.45 (SD = 4.68).

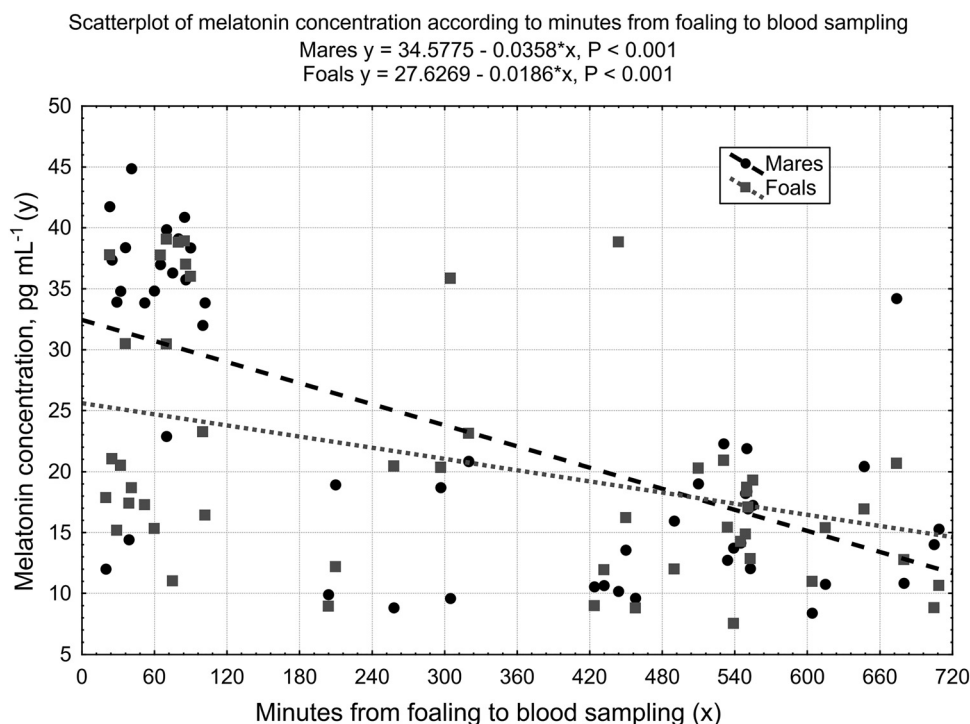


Fig. 3. Scatterplot of plasma melatonin concentration as a function of *minutes to blood sampling* according to the *generation*

This value falls in the range of 20–30, which reflects a "good" immunoglobulin G (IgG) supply in the mare's milk. All foals were clinically normal at birth and suckled colostrum without external assistance within two hours of birth.

## DISCUSSION

The plasma concentration of melatonin in the horse during the first 12 h after foaling, was determined successfully on a large dataset for the first time. The hypothesis that the concentration of melatonin in dams and their offspring is statistically identical was not confirmed. The adjusted melatonin concentration in mares at foaling was found to be  $34.58 \text{ pg mL}^{-1}$ . The adjusted plasma melatonin in new-born foals was found to be lower than the maternal concentration ( $27.63 \text{ pg mL}^{-1}$ ). However, by the end of 12 h, they decreased differently: the melatonin concentration dropped more in the mothers than in the offspring, regardless of the exact time of foaling. In human, twenty-four hours after caesarean section, plasma melatonin concentration has been found to decrease significantly below the concentration of the non-pregnant controls (Ejaz et al., 2021). These findings are consistent with the fact that the placenta is a major extrapineal source of increased plasma melatonin at delivery (Hardeland et al., 2017). Although there is no melatonin production in the rat placenta (Tamura et al., 2008), one may assume that it occurs in the horse as in humans (Ejaz et al., 2021) and loss of the placenta may explain the sudden drop in melatonin concentration in mares.

The present values of the progeny generation indicate that there is a moderate change in plasma melatonin concentration during this short period. Since mares and foals spent their first hours together in the same stall environment, this finding cannot be explained by the fact that only mares were exposed to more light after dawn. This observation may already be explained by the foal's independent melatonin secretion. However, it is also possible that the neuronal circuits transmit a light stimulus to the pineal gland, or its translation is not developed yet in neonates. Therefore, in this age group, external light reduces melatonin secretion and concentration less.

In a study (Kilmer et al., 1982) including foals from 1 week of age to the first 11 weeks, the post-partum melatonin concentration of the offspring has been found to remain unchanged, while mares demonstrated variable changes with time under natural conditions. In newly pregnant mares, increasing length of daylight in spring causes a further drop in the circulating melatonin concentration and an elevation in prolactin concentration.

Melatonin concentration did not change as a function of the *hour of foaling*. Therefore, it can be concluded that the diurnal cycle of melatonin concentration does not occur during the nocturnal hours of delivery; melatonin reaches its higher concentration at the moment of foaling regardless of its hour. This finding is supported by the fact that foaling and samplings took place continuously. There were several pairs of mares and foals that were sampled before midnight.

Similarly, stable nocturnal melatonin concentration in mares, sampled in March has been observed by Rapacz-Leonard et al. (2010). It started to decrease at sunrise.

Usually, the nocturnal melatonin peak in mares reaches  $23.52 \text{ pg mL}^{-1}$  in June and  $42.41 \text{ pg mL}^{-1}$  in December (Altinsaat et al., 2009). In our study, foaling occurred on average one week before the vernal equinox, so the expected night-time peak is the average of these two values (approx.  $33.0 \text{ pg mL}^{-1}$ ). The adjusted melatonin concentration in combined mares at foaling is higher ( $34.58 \text{ pg mL}^{-1}$ , especially if we consider the control mares only), even though the possible increase of melatonin concentration during the pregnancy was not monitored. According to a study in humans (Ejaz et al., 2021), maternal plasma melatonin increases during pregnancy, with concentrations in the third trimester and by a few days before parturition (caesarean section) being around 3-fold higher (about  $500 \text{ pg mL}^{-1}$ ) than in the first trimester, and also remarkably higher than in non-pregnant controls. Melatonin and its metabolites protect the mother-placenta-foetus connection by stimulating antioxidant enzyme activity (Reiter et al., 2009). As an anti-inflammatory substance, maternal melatonin has a key role in the development of the foetal central nervous system. It plays an essential role in the healthy development of the placenta. Melatonin, as a protective environment has been found to rescue the quality and developmental potential of ovine oocytes and cumulus cells after an *in vitro* ovary preservation (Sánchez-Ajofrín et al., 2022).

Taking into account the above-mentioned results, it could be supposed that a higher melatonin production may be related to the preparation for foaling thus ensuring the offspring's chances of survival. It may seem helpful, that in addition to the passive transfer of immunoglobulins (LeBlanc et al., 1992), the concentration of melatonin in the foal's blood is also included in the biochemical panel for assessing the health status of neonates (Austin, 2013; Pirrone et al., 2014).

Our second hypothesis was confirmed. The daytime light supplementation used at the end of pregnancy reduced the nocturnal melatonin concentration in the mares at foaling. The foals from these mares were born with a significantly lower supply of melatonin than control foals. Nevertheless, later on, the melatonin concentration of the controls shows a similar lower level as the animals receiving light supplementation 12 h after foaling. The effect of additional light may be considered harmful to the foal's development and general health state if subsequent performance is a function of the melatonin concentration at birth. This can be assumed despite the fact that no significant relationship was found between supplemental illumination and colostrum IgG content. In a recent investigation, the blue light supplementation decreased the birth height of foals but did not result in a difference in birth weight (Lutzer et al., 2022b). It is confirmed in the trial continued that no developmental differences in the same foals for the subsequent 12 months could be observed for any parameter between foals born from control or light-treated mares (Lutzer et al., 2022a). The need to monitor the melatonin concentration in the



offspring justifies further studies with special regards to the general health state of the foal.

In the case of the Thoroughbred, the administration of exogenous melatonin is clearly out of the question when light supplementation is performed for early re-breeding. It is noteworthy that the average time of foaling, which fell to March, can be considered relatively late in the Thoroughbred.

Thanks to carefully chosen sampling, it was possible to achieve a larger sample size than that published on similar melatonin research previously. This supports the reliability of our findings. However, the results would be more robust if samples were available not only from the early post-partum period but also from the entire course of gestation and could be compared with samples of non-pregnant horses kept in the same environment.

## ACKNOWLEDGEMENT

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