

## Original Article

# The effect of Equilume light masks on the timing of seasonal ovulation of Thoroughbred mares in South Korea

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**ABSTRACT** Advancing the estrous cycle of mares is an essential breeding strategy that is routinely conducted by Thoroughbred breeders to improve economic outcomes. For this purpose, Equilume light masks have been developed as an alternative to existing technologies such as artificial lighting or hormonal treatments because they are considered as valid as existing methods with additional animal welfare advantages. For example, with the Equilume light masks, horses can be let out into the pasture, whereas they have to be kept indoors during lighting treatment. Because the function of Equilume light mask on the estrous cycle of mares is influenced by environmental factors such as nutrition condition and temperature, Equilume light mask should be studied in various environments. The objective of the present study was thus to verify the effect of Equilume light masks on the onset of the estrous cycle in Thoroughbred mares in South Korea. Mares were randomly selected and separated into two groups at two Thoroughbred horse breeding farms. The mares in the treatment group were equipped with Equilume light masks from November 18 to February 10 the following year. The body condition, the number and size (> 35 mm) of uterine follicles, and the uterine horn score of the mares were assessed on January 6 and February 10. The body condition scores were not different between the two groups. The treatment with the Equilume light mask had no positive effects on developing follicles and the reproductive tract. In conclusion, the use of Equilume light masks did not influence the seasonal resumption of the estrus cycles in Thoroughbred mares in South Korea.

**Keywords:** Equilume light mask, estrus, horse, ovulation, seasonality

## INTRODUCTION

Horses are seasonal breeders, with the circannual rhythm and annual breeding season of mares driven by the photoperiod in association with melatonin secretion (Freedman et al., 1979). Mares become anestrus in winter and their reproductive cycle begins again in spring in the Northern Hemisphere (Nagy et al., 2000). Environmen-

tal conditions affect ovarian activity; for example, when spring transitions start, the longer day length triggers the resumption of the breeding cycle so that foals can be born and nursed during periods of warmer temperatures and in nutritionally richer pastures.

The estrous cycle in a mare is regulated by interactions between gonadal hormones (Brinsko et al., 2010). When the photoperiod increases, the retinas of the horse

receive light, which stimulates the pineal gland and suppresses the production of melatonin. Melatonin acts as an antagonist of gonadotrophin-releasing hormone (GnRH), which is secreted by the hypothalamus; GnRH stimulates the pituitary gland to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Once the production of melatonin in the pineal gland is suppressed due to the longer photoperiod, GnRH levels increase, and the secretion of LH and FSH is stimulated. This interplay between the hormones causes the estrous cycle to resume.

In the horse-breeding industry, a number of breeding technologies are employed to encourage foals to be born as early as January 1, the date that is regarded as the universal birthday for every horse in the Northern Hemisphere, while all Southern Hemisphere horses grow a year older on August 1. This is important because young Thoroughbreds are commonly categorized by their age at auction (i.e., foals, yearlings, and two-year-olds). Foals born early in the year are kept inside, which offers nutritional advantages and a lower risk of skeletal injury, while also having positive behavioral effects due to the fact that the foals come into contact with humans more often (Langlois and Blouin, 1997). Early-born foals are also expected to perform better than later-born horses of the same age (Langlois and Blouin, 1998), so breeders believe early-born foals are more likely to be auctioned off at a higher price.

Given the needs of the racehorse industry, past research has developed various methods for the artificial induction of ovulation in mares, including treatment with light (Nagy et al., 2000), pituitary extracts, GnRH, progestogens, dopamine antagonists, melatonin implantation (El-Mokadem et al., 2017), and recombinant gonadotrophin hormones (Meyers-Brown et al., 2011). Of these strategies, breeders routinely use artificial barn lighting to advance the breeding season for mares (Sharp et al., 1975). However, this requires significant time and labor because the horses need to be put into the stables and out into the pasture at appointed times daily, leading to increased costs for bedding, electricity, and workers.

Equilume light masks have been developed not only to provide the same effects as indoor lighting treatment but also to allow horses to be grazed outside. Blue light inhibits the secretion of melatonin in mares (Walsh et al., 2013), and the masks have been proven in recent studies to be as effective as indoor lighting in advancing the re-

sumption of the estrous cycle in mares, thus offering financial and animal welfare benefits (Murphy et al., 2014). In particular, animal welfare has become increasingly important in South Korea, so this new method could be helpful for breeders in this respect.

However, previous research on Equilume light mask has not been conducted under various environmental conditions. As such, the masks need to be tested in South Korea to ensure that they work as intended; in Korea, the winters are longer and colder, and horses are fed additional hay due to the lack of fresh grass during the spring transition, and these factors may make Equilume light masks less effective. This study thus aimed to investigate the effects of Equilume light masks on the onset of the breeding season in Thoroughbred mares in South Korea.

## MATERIALS AND METHODS

### Animals

All experimental procedures were approved by Kyungpook National University Animal Research Ethics Committee (2019-0166). The experiment was performed from November 18 to February 10 the following year (2016-2017) at two farms in Icheon and Sangju, Korea. The mean temperature in the two locations ranged from -8.8 to 12°C over the experimental period. Sixteen Thoroughbred mares were used, with ages ranging from 5 to 19 years (mean age:  $13.50 \pm 1.09$ ). Nine mares were randomly selected to the treatment group and seven to the control group. The ages ranging between control and treatment groups were from 10 to 19 (mean age:  $13.43 \pm 1.29$ ) and 5 to 19 (mean age:  $13.56 \pm 1.73$ ), respectively. All horses were allowed out into the pasture during the day and night, though they were occasionally put in individual stalls when it rained heavily. During the experiment, all mares were allowed to graze *ad libitum*. The mares were also fed Timothy hay and commercial feed (0.5% body weight/day), and were allowed access to water at all times.

### Equilume light masks

Nine commercial light masks (Equilume Light Masks, Equilume Ltd., Kildare, Ireland) were used for the treatment group in this study. The masks are made from synthetic fabric and have a waterproof plastic cup and a built-in battery. A single eye cup exposes the horses' right

eye to blue light.

### Experimental protocol

On November 18, all mares in the treatment group were fitted with the Equilume light masks. The LED light of the masks was set to turn on automatically at 16:30 pm and off at 23:30 pm each day. The mares in the treatment group wore the masks until February 10 the following year, while the horses in the control group went without masks.

### Body condition score

The body condition of the mares was graded using the scoring system proposed by Henneke et al., in which six regions of the body are visually evaluated and scored on a scale of 1 to 9 (Henneke et al., 1983).

### Rectal palpation and ultrasound sonography examination

The size of the follicles and uterine horn scores were assessed using ultrasound on January 6 and February 10. Ultrasound examinations were conducted to observe the number and size of the follicles and the uterine horn scores using a DP-6600 Digital Ultrasonic Diagnostic Imaging System (Mindray, Redmond, WA, USA), which has a frequency range of 2.0 to 10 MHz. Mares with follicle sizes > 35 mm and uterine horn scores = 3 were classified as having an activate estrous cycle (Hayes et al., 1985) (Table 1). The appointed veterinarians of each stud farm assessed the rectal palpation and ultrasonography, and the results were received from the stud farms.

**Table 1.** Ultrasonic morphology characteristic of uterine horn score

Uterine horn score	Estrus stage	Ultrasonic morphology
1	Diestrus	Endometrial folds not visible
2	Intermediate stage	Visible, but indistinct endometrial folds
3	Estrus	Distinct endometrial folds

**Table 2.** The body condition scores of the mares

Group/Date	November 18	January 6	February 10
Control	5.14 ± 0.50	5.36 ± 0.18	5.50 ± 0.24
Treatment	5.28 ± 0.24	5.50 ± 0.33	5.67 ± 0.28

The results are expressed as the mean ± SEM.

### Statistics

The body condition score was compared for the treatment and control groups using Student's t-tests and analyzed with repeated-measure analysis of variance (ANOVA) and verified by post hoc analysis using SAS (SAS Institute, Cary, NC, USA). For analysis of the size of the follicles (> 35 mm) and the uterine horn scores, Student's t-test was performed using SAS (SAS Institute, Cary, NC, USA). The results are given as the mean ± the standard error of the mean (SEM). The level of significance was set at  $p < 0.05$ .

## RESULTS

### Body condition score

The mean body condition score for the mares ranged between 4.0 and 7.0. There were no significant differences observed in the mean body condition score between the control group and the treatment group on November 18, January 6 and February 10 (Table 2).

### Size of the largest follicle

On January 6, the mean sizes of the largest follicles were  $13.86 \pm 3.06$  and  $11.89 \pm 1.06$  in control and treatment group, respectively. On February 10, the mean sizes of the largest follicles were  $12.00 \pm 6.82$  and  $13.00 \pm 4.05$  in control and treatment group, respectively. There was no significant difference in the mean size of the largest follicle between the treatment and control groups on either of the two assessment dates (Table 3).

### The number of mares with follicles > 35 mm

None of the mares exhibited follicles larger than 35 mm on either January 6 or February 10 (Table 4).

**Table 3.** The size of the largest follicle

Group	January 6	February 10
Control	$13.86 \pm 3.06$	$12.00 \pm 6.82$
Treatment	$11.89 \pm 1.06$	$13.00 \pm 4.05$

The results are expressed as the mean ± SEM.

**Table 4.** The number of mares with follicles > 35 mm

Group	January 6	February 10
Control	0	0
Treatment	0	0

**Table 5.** Mean uterine horn scores

Group	January 6	February 10
Control	0.14 ± 0.19	0.71 ± 0.63
Treatment	0.11 ± 0.13	0.89 ± 0.52

The results are expressed as the mean ± SEM.

### Uterine horn scores

There were no significant differences in the uterine horn scores between the treatment and control groups on the two assessment dates, with an average of less than 1 for both assessments (Table 5).

## DISCUSSION

Low-level Equilume light masks have been proven to be as effective as indoor lighting for advancing the breeding season of mares (Murphy et al., 2014). However, their effects on mares in different environments have not yet been reported.

In South Korea, the breeding season of the Thoroughbred mares is generally from March (Yang et al., 2004). Conventional lighting treatment is a common practice among South Korean breeders, and we hypothesized that Equilume light masks might affect the timing of the seasonal resumption of ovulation in Thoroughbred mares in South Korea. If the start of the breeding season can be advanced, mares have a higher chance of falling pregnant because they have more ovulation cycles during the estrus period, and breeders are more likely to produce early-born foals. In this study, however, there was no evidence that the mares in the treatment group had advanced estrous cycles on the February 10 assessment date.

The body condition score was not a significant factor in the failure to advance seasonal ovulation. There were no restrictions in terms of nutrition during the study. Thus the body condition scores of all mares were maintained at a sufficient level (> 5 out of 9) to stimulate the estrous cycle. Previous research has reported that nutritional factors, as assessed using body condition scores and fat thickness, affect reproductive seasonality in mares (Vecchi et al., 2010). With the onset of ovulation for those with a low body condition score ( $\leq 2.5$ ) being later than for other groups, while ovarian inactivity in winter is shorter for well-fed mares than in nutrient-restricted mares. Based on these studies, the mares in the present study received sufficient nutrition. Thus the body condition score was

not a major factor affecting seasonal ovulation.

Age may affect the reproductive efficiency of mares. One mare in the control group (14.29%) and two mares in the treatment group (22.22%) were older than 18 years. Previous research has indicated that age influences fertility in mares, with older mares (i.e.,  $\geq 19$  years) having a lower per-cycle pregnancy rate (Lane et al., 2016), slower ovulatory follicle growth, substantially smaller follicle sizes, and a lower number of follicles (Ginther et al., 2008). In particular, the small follicle size may lead to undesirable reproductive performance in older mares that have multi preovulatory follicles (Morel et al., 2010). Therefore, the age of the mares in the present study may have influenced the results. However, it does not explain the reason for the failure to advance seasonal ovulation in the younger mares in the treatment group.

Environmental conditions could also be a factor affecting ovarian activity, in particular the intensity of daylight, temperature, and access to green grass (Nagy et al., 2000). This experiment was conducted on two stud farms in Icheon and Sangju, which are located inland in South Korea. Geurin and Wang reported that both minimum and the maximum environmental temperatures influence the resumption of the estrus cycle in mares (Guerin and Wang, 1994). Also, it is generally known that the onset of the breeding cycle of winter anestrous mares was delayed by sudden cold spell in the spring transition (Allen, 1987). In contrast, Dini and coworkers reported that greater sunlight exposure affects the timing of the breeding season in mares during artificial photoperiod treatment, while differences in barn temperature had no effect (Dini et al., 2019). In Thoroughbred yearlings, an extended photoperiod activates gonadal hormones in both cold and warm climate (Suzuki et al., 2015). The association between environmental temperatures and the circannual breeding cycle in mares is not indisputable. Walsh and coworkers proved that Equilume light masks caused spring ovulation to arrive earlier (Walsh et al., 2013). However, these studies did not investigate the effect of sub-zero temperatures in February. To meet industrial needs regarding advancing the breeding season of mares, we expected the first ovulation to start in February. During our study, a cold wave hit Korea, thus it was still cold and windy at the end of the study period. The minimum temperature in February was  $-7.2^{\circ}\text{C}$  in Sangju and  $-12.8^{\circ}\text{C}$  in Icheon. On February 10, when the final rectal examination was conducted, the

mean daily temperature was  $-4.4^{\circ}\text{C}$  in Sangju and  $-5.8^{\circ}\text{C}$  in Icheon., and the mean daily wind speed was 15.5 km/h and 10.1 km/h, respectively. As a result, it can be assumed that the wind chill temperatures were significantly lower than those encountered in Walsh's study.

For reproductive management in mares, it is important to identify the optimal strategy for each horse. Common practice may not always be effective in every situation. Breeders should thus make an effort to examine the breeding history and underlying issues of each horse, while also considering its age and environmental factors.

Further studies are warranted to examine the efficacy of the Equilume light masks on the resumption of the seasonal ovulation with hormonal therapy or/and winter blankets to overcome the cold weather condition. It is recommended that subsequent research test the effectiveness of Equilume light masks with hormonal assays to determine hormonal changes and to analyze the mechanisms underlying these changes.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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