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# Efficiency of Equilume Light Mask on the Resumption of Early Estrous Cyclicity and Ovulation in Thoroughbred Mares


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**Running title:** Bluelight-mediated resumption of early oestrous cyclicity in horses in Korea

**Abstract**

Equilume light masks had no impact on hastening the resumption of estrous cyclicity in mares maintained in outdoor pastures on the mainland of South Korea due to the cold weather conditions. Jeju Island is a major horse-breeding site in South Korea and is warmer than the mainland during the winter season. Therefore, the primary objective of this study was to explore the efficiency of the Equilume light mask on the resumption of seasonal estrous cycles in Thoroughbred mares on Jeju Island. A total of 20 nonpregnant mares were randomly divided into the Equilume light mask (n = 9) and stable lighting (n =11) groups. The experiment was performed at seven different horse-breeding farms located on Jeju Island from November 15, 2020, to February 15, 2021. The mares were exposed to the respective lights from 16:00 to 23:00. Follicle size and uterine edema were measured by ultrasound scanning. Body condition scores were also monitored during the experiment. Statistical analysis was conducted using the SAS and SPSS software, and *p*-values of <0.05 were considered statistically significant. Two of the nine (22.2%) mares in the Equilume light mask group and three of the 11 (27.28%) mares in the stable lighting group were still cycling in December and January, which were considered as all-year-round cycling mares. On February 15, there was no difference between groups in the resumption of early seasonal estrus cycle, which was determined by follicles >25mm in addition to uterine edema. All mares in the Equilume light mask group and five of the eight mares (62.5%) in the stable lighting group had resumed cycling. Interestingly, six of the seven mares (87.5%) in the Equilume light mask and four of eight mares (50%) in the stable lighting group had already ovulated on February 15 (*p>0.05),* as determined by the presence of a recent corpus luteum. No difference was observed in BCS and uterine edema between groups (*p*>0.05). In conclusion, the Equilume light mask can be an effective approach to induce early seasonal estrus cycles of mares in Jeju Island, and it also enhances the efficiency of farm management by reducing labor.

**Keywords: Blue light, light mask, reproduction, early seasonal estrus cycle, mare, welfare**

## 1. Introduction

Horses are long-day seasonal breeding animals. During winter, ~85% of Thoroughbred mares cease normal cyclic activity and enter an anestrous period (1, 2). In mares, the circannual rhythm of reproduction is primarily regulated by photoperiodic changes, with the increase in day length preceding the onset of the breeding season (3, 4). It is well documented external factor that influences the circannual rhythm (5, 6).

During the anestrous period, mares typically exhibit minimal-to-moderate follicular growth and an absence of periodic ovulation as a consequence of lower gonadotropin secretion (7). Melatonin is one of the primary mediators in the regulation of gonadotropin-releasing hormone (GnRH) secretion (8). In mares, melatonin is synthesized and secreted by the pineal gland and its concentrations are strongly associated with the dark phase (9, 10). Melatonin secretion increases at the beginning of the dark phase and decreases rapidly at the end of the night (11). During winter anestrus, the extended melatonin secretion during long periods of darkness has an inhibitory effect on GnRH secretion, which causes a reduction in the secretion of gonadotropin, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) (12). During the spring, increasing day lengths causes a shortening in the duration of melatonin secretion (13), releasing its inhibitory effects on GnRH neurons and permitting the resumption of gonadotropin secretion and the restoration of cyclic ovarian activity (14).

Understanding seasonality and the mechanisms of hormonal interplay of mares has become important for the development of methods to advance the onset of seasonal breeding in the Thoroughbred breeding industry. This is important because the annual earnings from foals born in January and February are higher than those from foals born from March to June, thus influencing the economic benefits for horse breeders (15, 16). Therefore several clinical treatment, including the provision of artificial photoperiod, have been implemented in the industry to advance the estrous cycle of mares (8).

The majority of these treatments have focused on increasing the photoperiod, and it has been repeatedly demonstrated that an artificial, long-day photoperiod consisting of 16 hours of light and 8 hours of dark effectively advances the time of the first ovulation in mares (17). Clinically, various administrations such as recombinant equine FSH (18), progesterone (19), gonadotropin (20), dopamine antagonists (21), GnRH (22), and pituitary extracts (23) are used to stimulate an early onset of reproductive activity.

To remediate some inefficiencies of delivering artificial photoperiod and avoid the adverse effects associated with clinical drug administration, Equilume light masks have been developed. These masks consist of

a single headpiece, wherein a blue light is exposed to the right eye. Studies in Thoroughbred have shown that melatonin can be suppressed to daytime levels using low intensity blue light (468 nm) from light emitting diodes (LED) directed at a single eye (24) and Equilume light masks were shown to effectively advance the ovulatory season of the mare. To meet the industry requirements for early breeding, we previously conducted a study to evaluate the effectiveness of Equilume light masks in advancing seasonal reproductive activity of mares on the mainland of Korea. However, the induction of early reproductive activity in Thoroughbred mares was not observed on Feb 10 in this study (25). During the study, Korea experienced very cold weather, wherein the minimum temperature was –12.8℃ and mares had infrequent access to pasture grazing.

Our team speculated that the extreme environmental conditions and low ambient temperatures may have influenced the failure of the early onset of the breeding season of mares in the mainland of Korea. Therefore, we conducted the present study to investigate the efficiency of the Equilume light mask on the resumption of seasonal estrus in Thoroughbred mares in Jeju Island, a warmer region compared with the mainland and the primary location for Thoroughbred production in Korea.

## 2. Materials and Methods

### 2.1. Animals

The experiment was conducted from November 15, 2020, to February 15, 2021, at 7 different barns in Jeju Island. The temperature of the region ranged from -1.1℃ to 22.4℃. A total of 20 nonpregnant Thoroughbred mares, all aged between 3 to 17 years (mean age: 10.25 ± 1.00) were used for the experiment. All mares were randomly divided into the blue light group (n=9) and the stable light group (n=11). The mean age of mares was 6.33 ± 1.01 years in the blue light mask group and 13.45 ± 0.73 years in the stable light group. All mares were out on pasture to graze by day and were supplied *ad libitum* with hay and fresh water throughout the experiment.

### 2.2. Housing and management

The first 4 breeding farms (farms 1–2: six mares of the blue light group; farms 3–4: eight mares of the stable light group) were located (geographical coordinates: 33º 23′ 36.384″ N, 126º 16′ 0.6018″ E) within a radius of 10 kilometers in the west of Jeju Island, and the other three farms (farm 5: three mares of the blue light

group; farms 6–7: three mares in the stable light group; geographical coordinates: 33º 21′ 1.6344″ N, 126º 47′ 38.5764″ E) were located in the east within a radius of 13 kilometers in Jeju Island. Mares treated with blue light were maintained on pasture for 24 h per day throughout the study period, and no additional light was provided except the blue light and the natural photoperiod. Mares in the stable lighting group were pastured by day but brought into individual stables and exposed to stable white fluorescent light from 16:00 to 23:00 daily. All mares were stabled on occasions of inclement weather such as hail and heavy snow. Temperature data were recorded using a digital thermometer, and the mean temperature was calculated with the lowest and the highest temperature of the barns and the region. The daily temperatures recorded in the farms were compared

**2.3. Experimental design**

The seven owners of the seven breeding farms consented to participate in the experiment, and the information in the registry of all mares (age, parity, and foaling history) in Korea Racing Association was collected 2 weeks before the initiation of the experiment. Beginning on November 15, 2020, the blue light group of mares were fitted with Equilume light masks that deliver 50 lux of blue light (468 nm) to the right eye. Once activated initially at 16:00, the light mask automatically turned on at 16:00 and turned off at 23:00 each day without a requirement for battery change. On December 1, 2020, the mares in the stable light group were housed in individual stalls at night and stable light that was operated from 16:00 to 23:00 each day. Transrectal ultrasonography was conducted using a S6V Portable Digital Color Doppler Ultrasound System (Sonoscape, United Kingdom) for each group until the end of the study (December 28, 2020; January 20, 2021; and February 15, 2021).

**2.4. Body condition score**

The body condition score was evaluated for all mares by the same person using the Henneke system each month throughout the study period (26). Six body parts (neck, withers, back, tail-head, ribs, and behind the shoulders) were palpated and visualized and scored from 1 to 9, with 1 indicating poor body condition and 9 indicating an obese body condition

### 2.5. Criterion of mares' ovarian activity

The ultrasound scans were performed by a qualified veterinarian to check for ovarian activity in terms of the size and number of follicles and the presence or absence of uterine edema and corpora lutea. Estrous cyclicity was defined as the presence of follicles measuring ≥25 mm in diameter in conjunction with uterine edema indicative of estrus. Ovulation was determined by the presence of a previously unrecorded corpus luteum. The anestrus phase was determined as ovaries with follicles measuring <25mm in diameter and with no associated physiological characteristics typical of estrous activity. The characteristics of uterine edema were graded based on the visibility on a scale of 1–3: with 1 indicating endometrial folds not visible, 2 indicating visible but indistinct endometrial folds, and 3 indicating distinct endometrial folds.

### 2.6. Statistics

The SAS software (SAS Institute, NC, USA) was used to perform the statistical analysis using the general repeated model for repeated measures for the body condition score and the status of uterine edema. Results are shown as mean ± standard error of the mean (SEM). The SPSS software was used to perform to compare mean temperature between the barns using ANOVA to confirm differences in ambient temperatures between locations. Pairwise comparisons were conducted using chi-square for the statistical analyses for classification of estrus and anestrus and for the number of mares that had ovulated within each group on each date. Results are represented as the number of mares and calculated as the percentage. The level of significance was set at $p < 0.05$.



## 3. Results

### 3.1. Body condition score and the uterine edema score

The mean body condition score ranged from 4.0 to 6.0 for each group. There was no difference observed in the mean body condition score of the mares between groups. However, a difference was observed according to the time difference of each group. (Table 1). The uterine edema scores ranged between 1.0 and 2.0, with no statistical differences between the blue light group and the stable light group (Table 2).

### 3.2. Mare reproductive status

From December to January, in blue light treated mares, 2 mares were in estrus and 7 mares were in anestrus while in stable light treated mares, 3 mares showed estrus and 8 mares showed anestrus. 5 mares (2 from blue light and 3 from the stable light treatment group) were excluded for data analysis in February considering these mares are non-seasonal polycyclic. In February, all mares in the blue light treated group showed estrus, and 5 mares out of 8 showed estrus in the stable light treated group. The number of mares classified as being in the estrus or anestrus phase were not significantly different in December-January ($p = 0.795$) and February ($p = 0.186$). However, the number of mares showed estrus in the blue light treated group were higher than the stable light treated group. (Table 3).

### 3.3. Number of ovulating mares

Ovulation was observed in 4 out of 5 mares (both groups) in December to January but these animals were excluded in February's statistical data as mentioned earlier, keeping these mares non-seasonal polycyclic. In February, 6 out of 7 mares in the blue light treated group and 4 out of 8 mares showed ovulation in the stable light treated group. The proportion of mares that had ovulated in the blue light group and the stable light group was not significantly different in December-January ($p = 0.369$) and February ($p = 0.329$). However, the number of ovulated mares in the blue light treated group were higher than the stable light treated group. (Table 4).



### 3.4. Temperature

There was no significant difference in mean temperature between barns (Barn 1: 9.36℃; Barn 2: 9.32℃; Barn 3: 8.66℃; Barn 4: 9.12℃; Barn 5: 9.36℃; Barn 6: 8.12℃; and Barn 7: 8.92℃) (Figure 1). The mean temperature of Jeju Island was 9.4℃ (Figure 2).

## 4. Discussion

Advancement of the mare's breeding season using indoor lighting has become a standard management practice for Thoroughbred breeders. It is believed that the photoperiod is the dominant external factor that

188 influences the mare's reproductive rhythm, and providing additional light exposure during winter stimulates
189 ovarian activity in anestrous mares (27). This study was conducted to evaluate the efficiency of the Equilume light
190 mask on the resumption of early ovulation of Thoroughbred mares in Jeju Island, Republic of Korea. Our results
191 showed that the reproductively active period of a non-pregnant mare can be advanced using blue light exposure
192 to one eye from head-worn masks while mares are maintained at pasture and that this method is as effective as
193 maintaining mares indoors under stable lighting. This result is consistent with a previous study that showed a
194 resumption of early ovulation of Thoroughbred mares (28).

195 One limitation of the current study is the absence of a control group of mares maintained under natural
196 photoperiod conditions as these were not available. However, unpublished data collected by an experienced
197 veterinarian involved in this study and who has worked within the Thoroughbred breeding industry on Jeju Island
198 for more than twenty years suggests that the mean date of first ovulation in unlit mares occurs between late March
199 and early April. A similar experimental design to the current study was employed previously to evaluate light
200 therapies at advancing seasonal reproductive activity in mares

201 However, our study findings were different from the previous assessment of the effect of the Equilume
202 light masks on hastening the reproductive cycle parameters of mares (25). Results of the reproductive
203 examinations conducted in February showed that several mares exposed to blue light treatment exhibited estrus
204 cycles and ovulation in Jeju Island, whereas none of the mares previously examined at this time exhibited ovarian
205 activity when maintained at locations inland in Korea (25). The BCS for the mares were not significantly different
206 from that of the previous research, but the temperature was significantly different (29).

207 The role of additional external factors that can modulate the response of the mare to photoperiodic
208 manipulation has been described in the literature. The proposed factors include ambient temperature, body
209 condition, and quality of food (30, 31). The previous study aimed at advancing the early onset of the estrus cycle
210 of a mare using the Equilume light mask was conducted in mainland Korea (Sangju and Icheon), and the results
211 showed that the Equilume light mask was not effective in stimulating ovulations by February 10 (25). It was
212 hypothesized that the cold temperature was responsible for the failure of the effect of the Equilume light mask, as
213 the lowest temperature was –12.8℃ and the mean temperature remained 0.7℃ due to the cold weather front
214 during the time of the experiment. Hence, the present experiment was conducted on Jeju Island, which is warmer
215 than the mainland, and the results confirmed the effectiveness of the Equilume light mask. During the present
216 study, the mean temperature of barns and Jeju Island were 9℃ and 9.4℃ respectively, and the minimum

temperature was also -2.8℃ which lasted at the most for 1–2 days during the experiment. Previous research supports that the onset of reproductive activity is closely related to the minimum and maximum environmental temperature (30). It has also been reported that the spring transition is slowed by cold weather (32). Therefore, the ambient temperature might have played a role as an additional external factor in advancing the resumption of estrus cycle in mares. Several studies have attributed a role to BCS, quality of feed in the regulation of reproductive efficiency. Good BCS was found to have a positive effect on the early onset of the mare's breeding season (33). Mares with a poor body condition score of <5 have a longer interval to first ovulation than mares with better body condition (6). Moreover, BCS is closely related to nutrition, as the quality of feed may advance the onset of the mare's cycle activity (31). Another study reported on the advanced onset of the breeding season in mares provided with supplemental feed while grazing (34). In this study, supplemental feed was provided to all mares during grazing in the pasture. Therefore, based on these studies, mares in the blue light group and the stable light group maintained a BCS of ≥ 5, indicating that the quality of the feed was sufficient and the body condition was ideal for the reproductive cycle.

## 5. Conclusion

Equilume light masks can be an effective approach to induce the early seasonal estrus cycle of Thoroughbred mares in Jeju Island, Korea. Their use also enhances the efficiency of farm management by reducing labor and providing improved animal welfare by permitting the outdoor maintenance of mares in groups so that natural behaviors can be expressed.

## Competing interests

Barbara A. Murphy is the Founder of Equilume Ltd, a spin-out company deriving from her research program as assistant professor at UCD and is a member of the company's Board of Directors. Dr. Murphy is a shareholder in Equilume Ltd. The light mask used in the presented study is a commercially available product with the following patents: AU2016231515, GB2504244, GB2549682, HK1245690, US9,839,791, US10,926,101.


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## Author's Contributions

Seongmin Kim contributed to conceptualization, methodology, formal analysis, data curation, and original draft preparation. Minjung Yoon, Barbara A. Murphy, and Heejun Jung contributed to methodology, data analysis and review and editing of the manuscript.

## Ethics approval and consent to participate

All procedures in this study were approved by the Animal Experiment Ethics Committee of Kyungpook National University (permit number: 2020-0119-1).

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332

333

334 **Table 1**

335 The mean body condition score of the mares

| Group | November | December | January | February |
|---|---|---|---|---|
| Blue light (n=9) | $4.94 \pm 0.10^{a}$ | $5.38 \pm 0.07^{b}$ | $5.5 \pm 0.08^{b}$ | $5.38 \pm 0.07^{b}$ |
| stable light (n=11) | $5.09 \pm 0.16^{a}$ | $5.4 \pm 0.06^{b}$ | $5.45 \pm 0.04^{b}$ | $5.36 \pm 0.07^{b}$ |

336 The results are expressed as the mean ± SEM, Different superscripts indicate statistical difference ($p < 0.05$)

337

338 **Table 2**

339 The mean uterine edema score of the mares

| Group | December | January | February |
|---|---|---|---|
| Blue light (n=9) | 1.44 ± 0.29 | 1.67 ± 0.29 | 2.11 ± 0.30 |
| stable light (n=11) | 1.83 ± 0.29 | 1.41 ± 0.19 | 1.63 ±0.24 |

340 The results are expressed as the mean ± SEM

341

342 **Table 3**

343 Comparison of the number of mares determined to have estrus or anestrus phase

| Group | December | | | January | | | February | | |
|---|---|---|---|---|---|---|---|---|---|
| Reproductive status | Estrus | Anestrus | $\chi^2$ (*p*) | Estrus | Anestrus | $\chi^2$ (*p*) | Estrus | Anestrus | $\chi^2$ (*p*) |
| Blue light (n = 9) | 2 | 7 | .067 (.795) | 2 | 7 | .067 (.795) | 7 | 0 | 3.367 (.186) |
| Stable light (n = 11) | 3 | 8 | | 3 | 8 | | 5 | 3 | |

344

345

346 **Table 4**

347 Comparison of the number of mares determined to have ovulated

| Group | December | | | January | | | February | | |
|---|---|---|---|---|---|---|---|---|---|
| Reproductive status | ovulated | not ovulated | $\chi^2$ (*p*) | ovulated | not ovulated | $\chi^2$ (*p*) | ovulated | not ovulated | $\chi^2$ (*p*) |
| Blue light (n = 9) | 1 | 8 | .808 (.369) | 1 | 8 | .808 (.369) | 6 | 1 | 2.222 (.329) |
| Stable light (n = 11) | 3 | 8 | | 3 | 8 | | 4 | 4 | |

348

## Figure 1

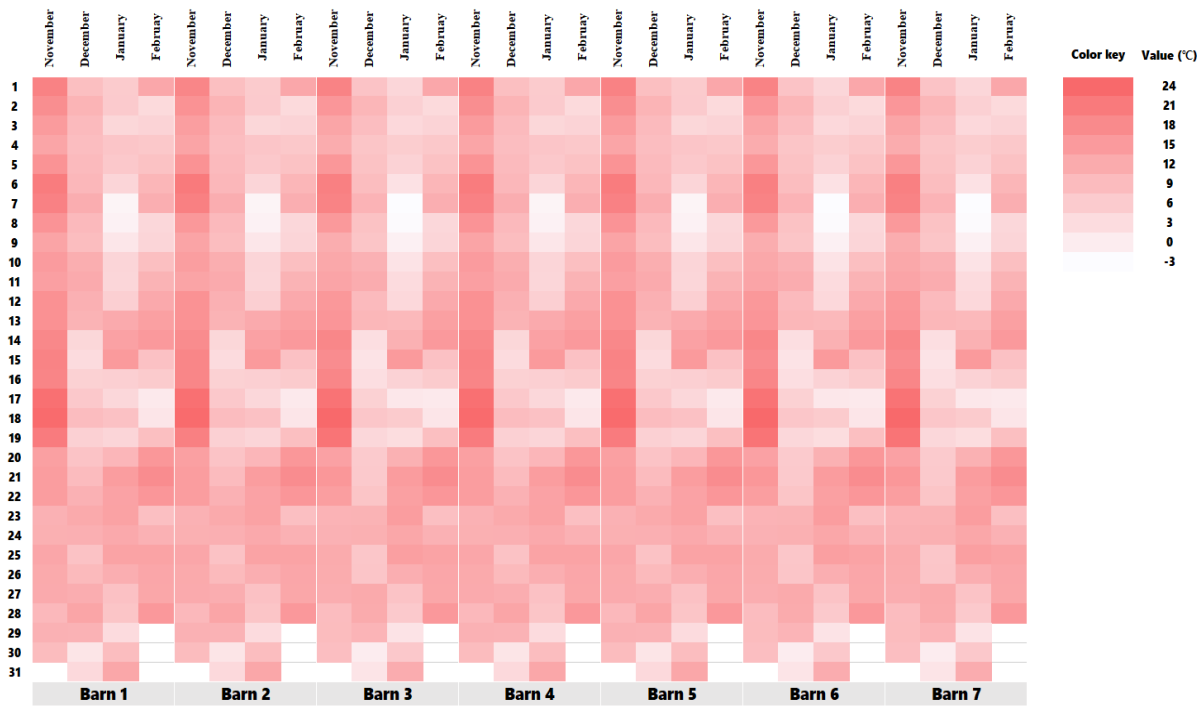


Figure 1. Heatmap profile showing the daily mean temperature in each barn. Dark red indicates the maximum daily mean temperature and white indicates the minimum mean temperature. The values are expressed in degree celsius.

**Figure 2**

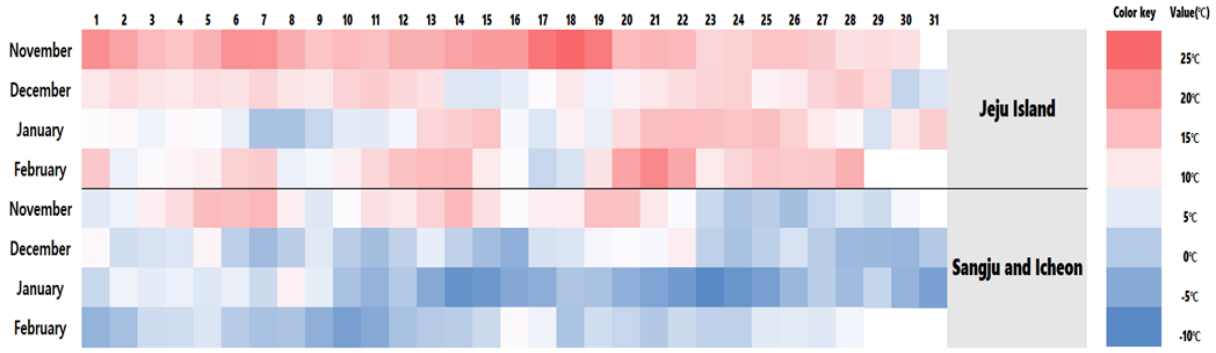


Figure 2. Heatmap profile showing the daily mean temperature in Jeju Island and Sangju and Icheon. Dark red indicates the highest daily mean temperature and dark blue indicates the lowest mean temperature. The values are expressed in degree celsius.