

A customised LED lighting system utilising daytime polychromatic white light and night-time red light influences testosterone levels and semen parameters in Sport Horse stallions

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Application Sport Horse stallions are used for breeding purposes throughout the year, but are often busiest between Jan and Apr. The lighting regime evaluated in this study improved fertility parameters by increasing Total Sperm Number and testosterone levels early in the breeding season and could represent an effective management tool for stallion management.

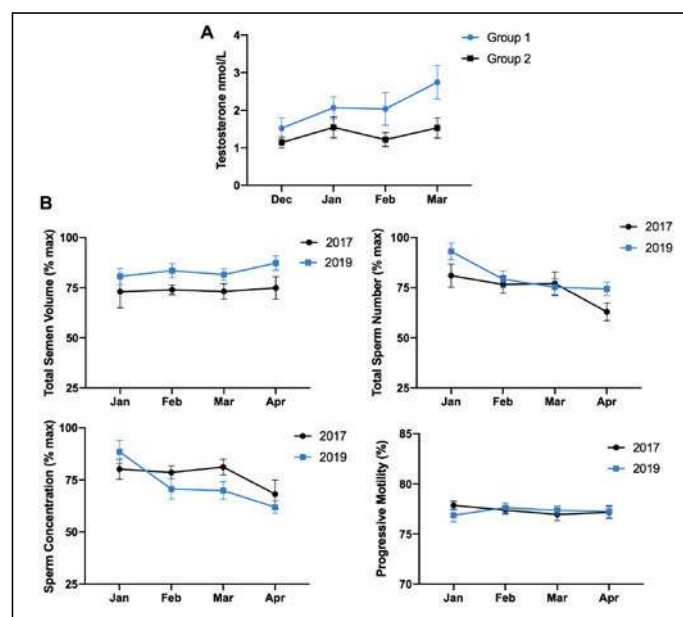
Introduction In horses, retinal photoreception records the annual cycle in day length and synchronises peak reproductive activity with the lengthened photoperiods of late spring and summer via modulation of daily pineal melatonin production. Short-wavelength blue light effectively suppresses melatonin and can be used to manipulate seasonal reproduction in mares (Murphy et al., 2014). Reproductive and behavioural characteristics in stallions also undergo seasonal variations related to photoperiod length (Pickett et al., 1976; Clay et al., 1987). The present study aimed to assess the effects of a customised LED lighting system on fertility parameters in stallions between Dec and Apr.

Material and methods 22 warmblood stallions from a commercial stud were blocked for age and randomly allocated to one of two groups: Group 1 (Treatment; n=11; mean age = 10.5±5.8yr) were housed under a customised LED lighting system and received 17.5h of blue-enriched polychromatic white light by day (peak wavelength 454 nm, >200 lux), 6.5h of dim red light by night (625 nm, 10 lux) with 20 min gradual light transitions at dawn (6am) and dusk (11.30pm) from Oct 28th, 2018. Group 2 (Control; n=11; mean age = 9.6±5.4yr) received 12h of standard fluorescent light by day, no light at night and abrupt transitions at dawn (6am) and dusk (6pm). At the start of each month from Dec 2018 until Apr 2019, a single morning blood sample was taken from each stallion and analysed for testosterone (SYNLAB, Germany) using the LCMS method (Wang et al., 2014). Semen ejaculates were evaluated from Group 1 stallions from Jan to Apr 2019 and monthly means for Total Semen Volume (TSV), Sperm Concentration (SC), % Progressive Motility (PM) and Total Sperm Number (TSN) were calculated and compared to those of Group 1 stallions during the same time period in 2017 when housed under control conditions. Recording of semen data began once sperm viability >50% had been determined using a NucleoCounter device (SP-100, Chemometec). TSV (mL) was determined by weighing the ejaculate on a calibrated scale (1g = 1mL). %PM was analysed visually under a microscope by an experienced technician. SC (million/mL) was determined using the NucleoCounter. TSN (billion) was determined by multiplying SC and TSV. To account for individual variability, values for TSV, TSN and SC were normalized within stallion as a % of maximum. Data for all parameters were found to be normally distributed using the Shapiro-Wilk test for normality. Data were analysed using a mixed effects analysis with repeated measures in Prism Version 8.01 for macOS (Graph Pad Software, USA). For testosterone; month, treatment and month x treatment were considered fixed effects and stallion within group was

considered a random effect. For semen parameters; month, year and month x year were considered fixed effects and stallion, stallion by month and stallion by year were considered random effects. Sidak's multiple comparisons tests were performed where appropriate and P<0.05 was considered significant.

Results An effect of month (P<0.05) and treatment (P<0.05), but no month x treatment interaction was observed for testosterone levels with Group 1 stallions exhibiting higher levels across all months. There was an effect of year on TSV (P<0.05) with higher levels in 2019, but no effect of month or month x year interaction. There was an effect of month on TSN (P<0.01) and an effect that approached significance for year (P=0.055) and month x year interaction (P=0.054). Sidak's multiple comparisons test revealed higher values in Jan compared to Apr in 2019 (P<0.05). An effect of month only (P<0.01) for SC was observed. There was no effect of month, year or month x year interaction for % PM. Results are described in Figure 1 and data represented as means ± SEM.

Figure 1 A) Serum testosterone levels in stallions exposed to 17.5h daily blue-enriched polychromatic LED light (Group 1; n=11) or 12h fluorescent light (Group 2; n=11) from Oct 28th. B) Semen parameters for TSV, TSN, SC and %PM in Group 1 stallions during the months Jan-Apr 2017 and 2019. All data are presented as means±SEM.



Conclusions A customised LED lighting system providing extended daily blue-enriched polychromatic light and red light at night increased mean testosterone levels in treated stallions compared to age-matched control animals between Dec and Apr. Marked increases in TSV and TSN were observed in treated stallions compared to previous historical data from the same animals. Modifying the method of light exposure may represent a simple management tool for improving fertility parameters in breeding stallions.

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